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THE PHYTOTOXICITY OF DESIGNATED POLLUTANTS

First Annual Report

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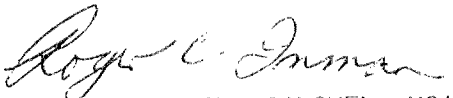

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Fumigations	HF	Light fuel	Shale fuel	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The phytotoxicity of gaseous hydrogen fluoride (HF) and jet fuel in vapor, spray and liquid states was investigated. The HF fumigation chamber and generating system was made operational, safe, and reliable. Plants exposed to 20-minute doses of HF gas developed typical foliar wilt, glazing, and necrosis dependent on gas concentration, plant species, and environment. Pictorial keys were constructed to aid in consistent grading of injured plants. Visible foliar response of six species to HF gas was comparable to their response to HCl gas, but at smaller concentrations. Seed development was inhibited after exposure to HF under certain

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conditions. Literature concerning the effects of hydrocarbon fuels on plants was reviewed. Plants exposed to sprays and vapors of JP4 jet fuel developed water-soaked lesions and foliar necrosis depending on amount of fuel applied. The response of seeds to liquid fuel was tested. A toxic substance in jet fuel moved short distances horizontally across flats and vertically down through columns of soil. Watering or airing soil treated with jet fuel decreased phytotoxic response. Species differed in tolerance to the fuel. Initial studies indicated that shale-derived jet fuel was biologically more toxic than the same type of fuel derived from petroleum.

SUMMARY

The phytotoxicity of gaseous hydrogen fluoride (HF) and jet fuel in vapor, spray, and liquid states was investigated.

The HF fumigation chamber and generating system was made operational. Safety and reliability of the HF generator were confirmed. Plants exposed to 20-minute doses of HF gas developed typical foliar wilt, glazing, and necrosis which was dependent on gas concentration, plant species, and, to a lesser extent, environmental conditions. A uniform coding system using pictorial keys aided in consistent grading of large numbers of injured plants. The visible foliar response of six species to exposure to HF gas was measured and found to be comparable to the response of these species to hydrogen chloride (HCl) gas, albeit at smaller concentrations. Development was inhibited after exposing seeds to HF gas under certain conditions.

We reviewed the body of literature regarding the effects of hydrocarbon fuel on plants. Systems were developed for exposing plants to sprays and vapors of JP4 jet fuel. Water-soaked lesions and foliar necrosis increased with the amount of fuel applied. The general susceptibility and tolerance of certain species according to the literature was verified for the jet fuel. Further studies concerned the response of seeds to liquid fuel. A toxic substance present in jet fuel moved short distances horizontally across flats of soil and vertically through soil columns. The effect of fuel or of toxic substances in the fuel varied from a lack of seed germination to inhibition of seedling growth. Water flushing or airing (aging) soil treated with jet fuel decreased but did not remove all phytotoxic products. Species differed in tolerance to the fuel; of the species tested, monocots were more sensitive. Initial studies indicated that shale-derived JP4 jet fuel was biologically more toxic than the same type of fuel derived from petroleum. Only one source of each fuel type was available to test and differences between batches probably exist.

PREFACE

This is a report of work performed by members of the Statewide Air Pollution Research Center, University of California, Riverside during the period from July 1, 1980 to June 30, 1981. The project was sponsored by Air Force Contract F-33615-80C-0512 to the University of California, Irvine.

The authors appreciate the cooperation and aid of Air Force Contract monitor Major James M. Livingston, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. The comments and suggestions of Drs. T. T. Crocker, H. Hodge, and J. D. MacEwen of the Scientific Advisory Board Executive Committee have been appreciated. We also acknowledge the aid of E. A. Allingham, A.B. Baudoin, Ph.D., H. E. Stone, G. Carrillo, E. C. Smith, J. Y. Lin, T. MacRunnel, M. A. Lardner, and H. C. Carpelan during portions of this project. Ms. Allingham and Mr. Smith conducted much of the fluoride work; Dr. Baudoin and Mr. Stone each were responsible for portions of the jet fuel investigations. The information and advice supplied by Major J. C. Allen, Occupation and Environmental Health Laboratory, Brooks Air Force Base, Texas, on soil bioassay techniques, was valuable.

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INTRODUCTION

This project is a continuation of a previous Air Force sponsored contract (AMRL AF F33615-76C-5005) in which our research group investigated the effects of certain Air Force-related pollutants on terrestrial vegetation. In previous annual reports under the former contract (Granett and Taylor 76,77,78,79,80a), we detailed the theoretical impact of HCl gas and aluminum oxide particles generated by solid rocket engines on plants in the vicinity of a space shuttle launch at Vandenberg Air Force Base.

The present contract again addresses environmental toxicology concerns, specifically the impact of Air Force operations on vegetation. Our research has had two main thrusts. One research area, a direct carryover from the HCl work, concerns gaseous hydrogen fluoride (HF). Certain rockets are powered by fuel which releases large quantities of HF gas. Plant sensitivity to HF has long been recognized (Treshow and Pack, 1970). Fluoride can be translocated within the plant and can accumulate in areas until toxic

levels are reached (Jacobson et al., 1966). Previous research was generally limited to small concentrations of HF present in the atmosphere for extended periods, such as releases from smelters and other industrial sources (National Academy of Sciences, 1971). Rockets create instantaneous and large concentrations of pollutants which usually dissipate soon after being released (Dawborn and Kinslow, 1976; Heck et al., 1962; Nadler, 1976). We limited our present HF studies to comparing the sensitivity of certain plant species and seeds to 20-minute exposures of HF.

Another major thrust of our research activities concerned the effect of hydrocarbon fuels on plants. Several considerations prompted that we study standard jet fuel, JP-4. Little background information is available on the environmental effects of this material, and future hydrocarbon fuels will be derived increasingly from shale rather than petroleum sources. Although fuels from the two sources perform identically, biologically significant differences may exist and should be documented.

Jet fuels can reach living plants in many ways through Air Force operations. Fuel is released to the environment by way of jettisoning fuel from flying aircraft (Clewell, 1980), spilling fuel during ground transport, and releasing fumes from fuel reserves. Plants can be exposed to fuels as vapors, aerosol sprays, or drenches. Fuels are complex mixtures of many compounds, some of which have greater phytotoxicity than others (Van Overbeek and Blondeau, 1954).

A literature review was prepared documenting knowledge of the phytotoxicity of hydrocarbons. Experimental work included constructing and testing relatively simple systems for exposing plant to fuels as sprays and vapors. Additional work has concerned fuel as a soil drench, particularly on how fuels move in soil and the sensitivity of plants and seeds to this form of contamination.

MATERIALS AND METHODS

EXPOSURE EQUIPMENT

HF Exposure Chamber

Two metal-framed continuous stirred tank reactor (CSTR) chambers (Heck et al., 1978) were used for exposing all material to HF gas. These units (Figure 1), previously described by Granett and Taylor (1978, 1981), were each 1.21 m high and 1.05 m in diameter. The two units were covered with 3 mil Tedlar film and had paddles which rotated at 120 rpm to mix the gases. Gases were exhausted through ca. 120 6-mm diameter holes in the floor of each chamber. A single exhaust fan drew air from both chambers through a 1 x 0.6 x 0.6 m filter box containing fluoride-absorbing calcium carbonate ("crushed oyster shell" pellets) supported in a plastic mesh (Figure 2). The exhaust manifold terminated outside and above the 4.6 x 6.2-m glasshouse which housed all the fluoride exposure facilities (Figure 3).

Each chamber has a door frame the height of the chamber. To prevent excessive gas loss during plant removal, a 6-mil vinyl sheet was placed on the inside of the chamber to reduce the size of the door opening.

HF Generating Systems

The HF generator consisted of two Orion syringe pumps forcing aqueous hydrofluoric acid solution from a specially constructed 40-ml Teflon syringe into 1.6-mm Teflon tubing (Figure 4). The tubing entered a large industrial oven bringing the acid to a Teflon T-union (Figure 5). Carrier air, heated to ca. 100°C in 20 feet of 6-mm diameter copper tubing coiled in the oven, entered the T-union volatilizing the solution and producing HF gas. The gas moved through a 4.6-m coil of 6-mm Teflon tubing inside the oven before continuing through 6-mm tubing encased in an insulated and heated PVC tube to the chamber intake manifolds.

Two syringes were machined from solid Teflon stock (Figure 6). They performed smoothly without the HF-induced etching and the binding and aging inherent in glass or plastic syringes. Miniature liquid-gas chromatography valves (Hamilton Instruments Inc., Reno, Nevada) were fitted to the syringe to facilitate filling and injecting. Both syringes could be mounted on one syringe pump; alternately, each pump could advance individual syringes. The syringes, syringe pumps, and aqueous HF were housed in a wooden hood adjacent to the generating oven (Figure 7). The hood was equipped with an exhaust fan which directed hood air to the filter box.

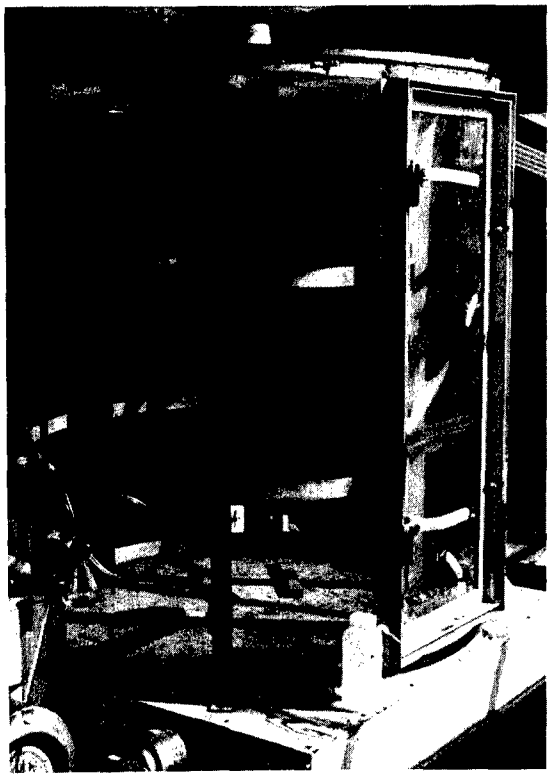
The carrier air system was modified from the use of pressurized nitrogen gas in tanks to one in which a 0.25-hp single cylinder air pump supplied compressed air. The air was dried in a Gilbarco heatless air drier and its flow was regulated (Figure 8). The compressed air reservoir tank was maintained at about 15 psig and output pressure at a pancake regulator was about 3 psig at a flow rate of 12 liters per minute per chamber. A safety switch installed in the exhaust tube activated an alarm when exhaust air flow fell below a safe minimum.

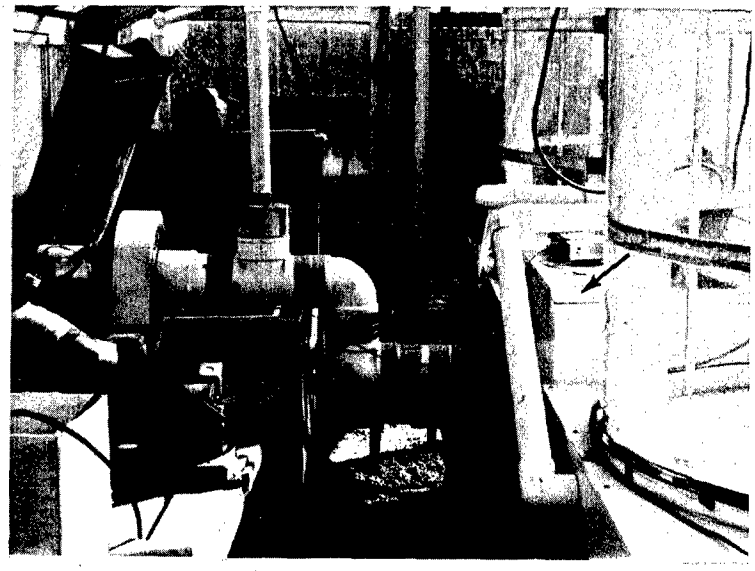
Aluminum conduit extending from the oven to both chambers contained the Teflon tubing carrying HF gas. The conduit was wrapped with heat cable over Tedlar film to prevent condensation. Foam insulation made for steam pipes enclosed the conduit and heat cable system. The cable was controlled by an electronic regulator set at 40°C. A thermistor probe from the regulator was inserted in a hole in the aluminum conduit.

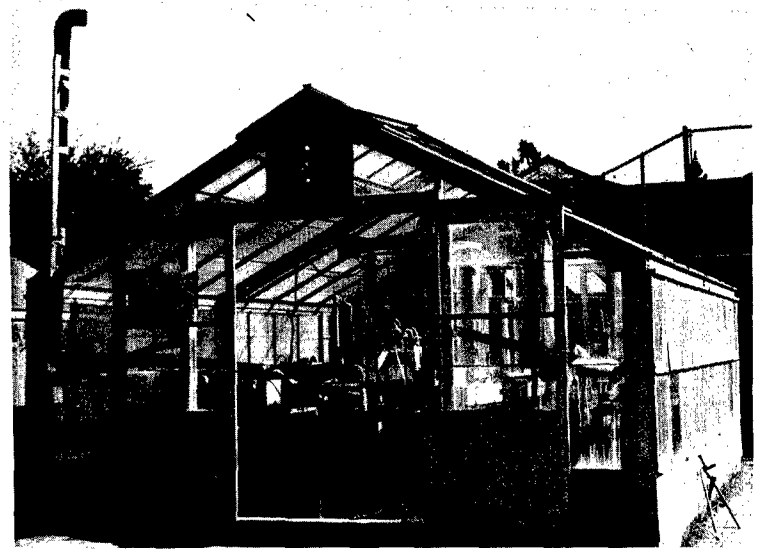
HF gas concentration could be adjusted by changing solution concentration, syringe pump speed, carrier gas flow rate, or flow rate through the chamber. In practice, all factors except the HF acid concentration were kept constant.

Equipment for Exposing Plants to Jet Fuel

The fuel experiments involved diverse techniques arising from the need to develop methods appropriate for experiments with a (typically) non-gaseous pollutant. Initial spray experiments were conducted outside glass-





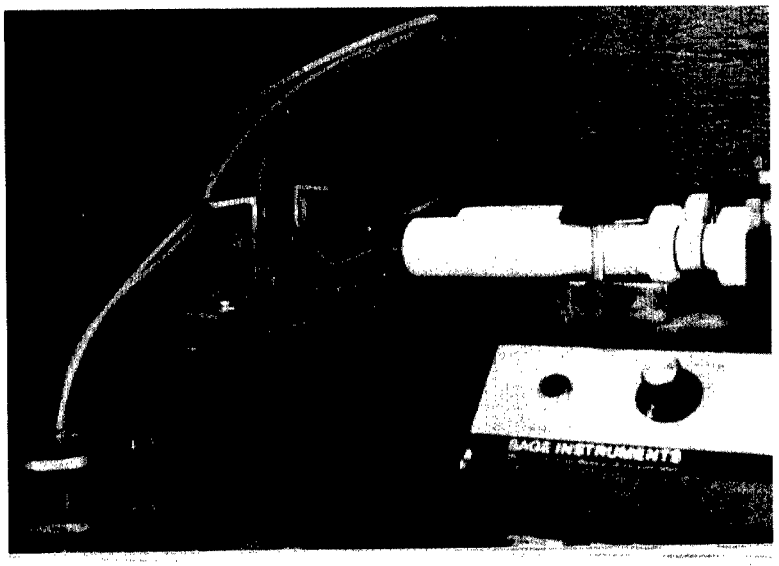


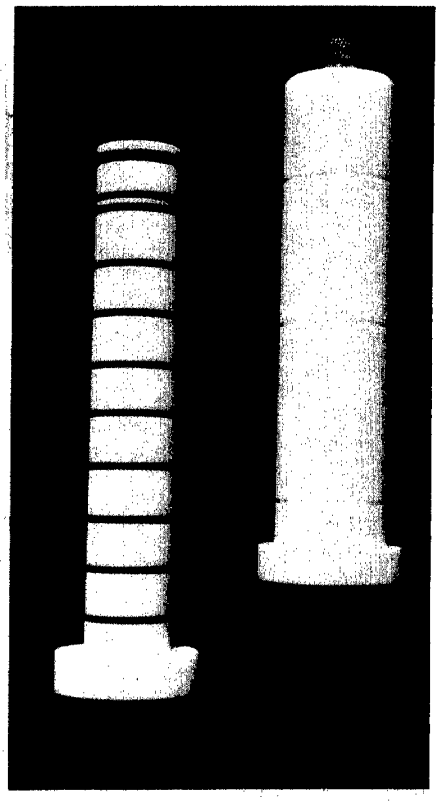
FACILITIES FOR HF EXPOSURES.

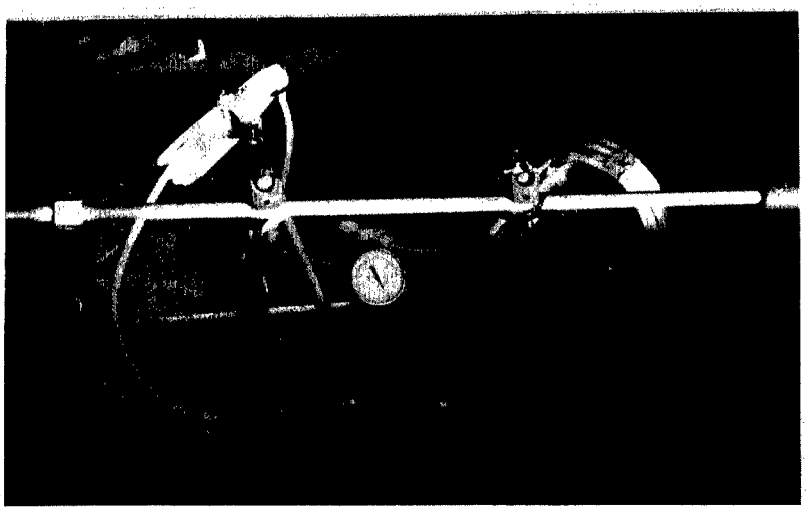
Figure 1 (left). Continuous stirred tank reactor (CSTR) for exposing plants.

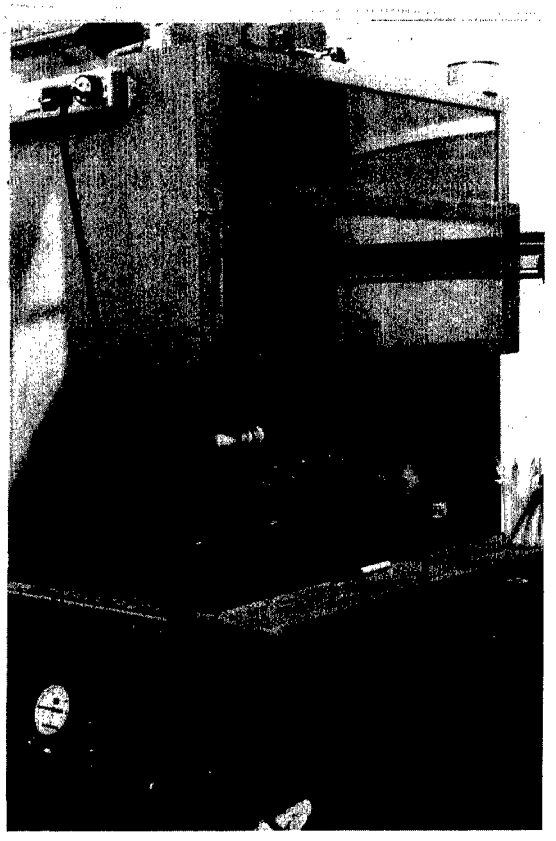
Figure 2 (upper right). Exhaust fan (left) connects chamber base to exhaust by way of limestone-pellet filter box (arrow).

Figure 3 (lower right). Glasshouse contains fumigating, generating, and sampling equipment.









HF GENERATION SYSTEM.

Figure 4 (upper left). Syringe pump feeds acid in two Teflon syringes into three-way valves.

Figure 5 (lower left). Generator oven open to show valves to control carrier air flow, Teflon tees where hot air vaporizes HF acid, and coils of Teflon tubing to smooth flow of HF gas.

Figure 6 (upper right). Teflon syringe with Luer-lock connector. O-rings seal syringe and act as 5 ml markers.

Figure 7 (lower right). Hood with syringe pump and syringes. Air dryer canisters (right), storage tank, and regulator (left) are visible below hood.

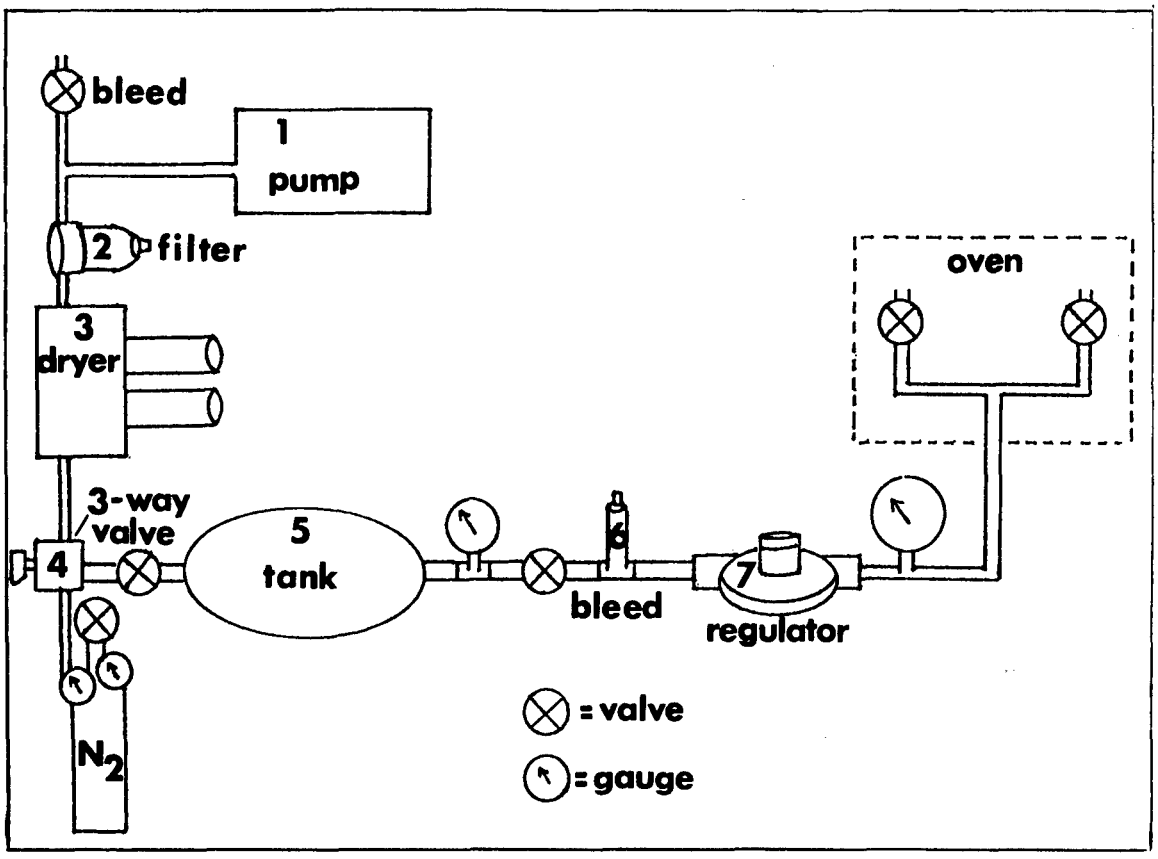


Figure 8. Diagram of the carrier gas system for the hydrogen fluoride exposure apparatus. Numbers refer to components: (1) Bell & Gossett air compressor, Model SY05-1; (2) Speedaire line filter, Model 4Z034; (3) Gilbarco heatless air dryer, Model HF-200, six-inch cylinders, no. 29 orifices; (4) Whitey three-way valve; (5) Low-pressure oxygen tank, 2100 in^3, 125 psi; (6) Speedaire bleed valve, Model 2X947A; (7) Matheson pancake regulator, Model 71-5.

houses or chambers. Later studies involving either sprays or vapors were conducted in a rectangular Lexan (plexiglas) chamber measuring 0.9 x 0.75 x 1.0 m equipped with a sliding front door (Figure 9). A spinning-disk pesticide applicator (Mini-ULVA, Micron Syringes Ltd., Houston, Texas) was installed in the chamber and liquid fuel was delivered to the sprayer with a calibrated syringe (Figure 10).

Fuel vapor was generated by installing a glass rod across the inside of the Lexan chamber and applying liquid fuel to absorbent paper suspended from the rod. The chamber was closed after the fuel was applied.

Liquid jet fuel was applied as a drench to the soil surface or was mixed thoroughly into a known volume or weight of soil. Soil mixing was accomplished by placing 500 g of soil into a one-gallon can, adding fuel, sealing the can, and rolling it.

Horizontal fuel movement was detected using flats of soil and vertical movement was tested within columns of soil in PVC tubes. In both cases, fuel was applied on the soil surface and seeds were used for bioassays.

PLANT PRODUCTION

All materials exposed to pollutants as plants or seedlings were grown in a glasshouse supplied with charcoal-filtered air, steam heat, and evaporative coolers. The plants were grown from seed to specific ages depending on the species (Table 1). Plants were grown in 350-ml styrofoam cups, 4-inch plastic pots, or wooden flats. Most plants were grown in UC Soil Mix II, a modification of Mixture B described by Matkin and Chandler (1972) as useful for growing most plants under glasshouse conditions (Table 2), and fertilized with Hoagland's solution (Hoagland and Arnon, 1950).

Plant species were chosen for specific reasons including horticultural type, ease of growth, and physical characteristics such as size, shape, and number of leaves. Both monocots and dicots were tested. Representatives of field, vegetable, and garden species were exposed to the toxicants. Some species tested earlier for sensitivity to HCl were included in HF studies so the phytotoxicity of the two gases could be compared.

Tomato seeds were used in several HF investigations since this species had been useful in the HCl work (Granett & Taylor, 1980b). Radish seeds were chosen because their resistance to HF contrasted with the susceptible tomato. Seeds of different species were exposed to fuel, and were usually germinated on moistened filter paper disks in closed Petri dishes in a dark drawer in the laboratory at 22-25°C. In other tests, seeds were sown in soil and incubated in the glasshouse. Seed germination, seedling emergence, and seedling lengths were measured and recorded.

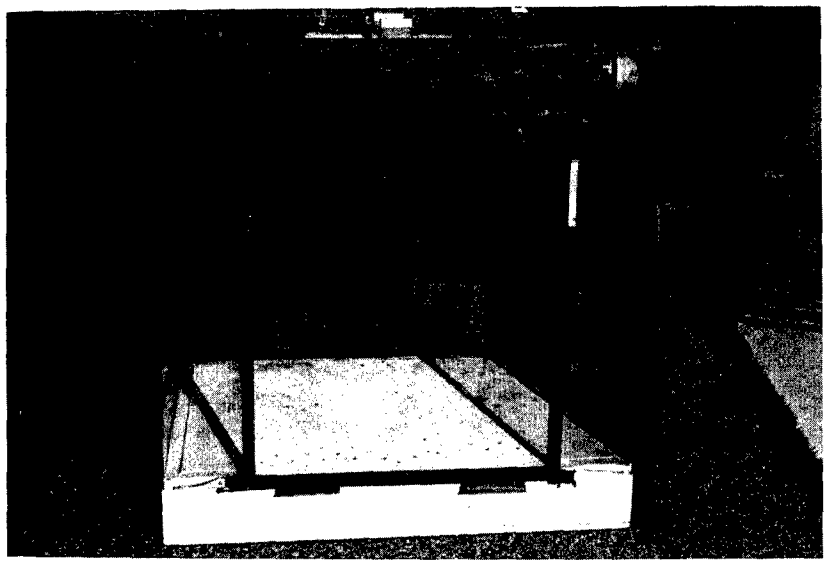


Figure 9. Lexan chamber for exposing plants to jet fuel.

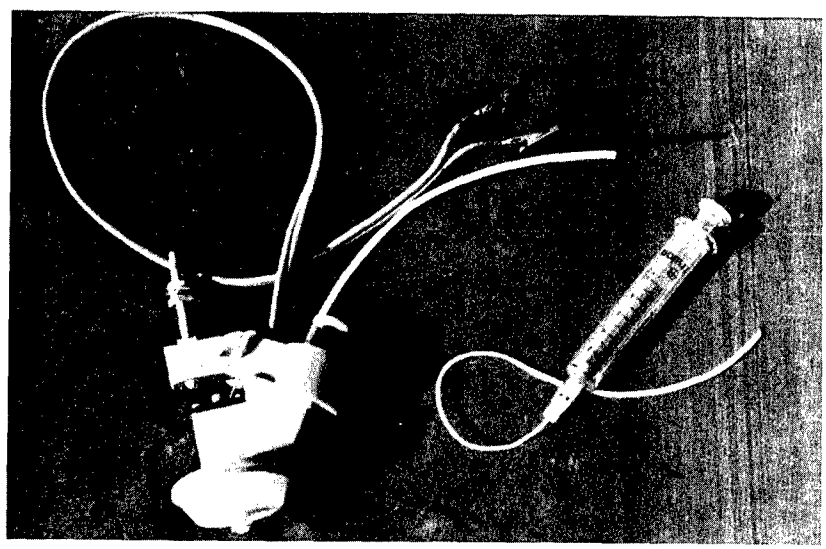


Figure 10. Battery-operated spinning-disk mist applicator (left) installed in chamber; syringe controls fuel entering mist applicator.

CHEMICALS

Hydrogen fluoride solutions for injection were prepared by diluting 52% hydrofluoric acid with distilled deionized water.

Two formulations of JP-4 jet fuel were supplied by the Air Force Aerospace Medical Research Laboratory. One was derived from petroleum (JP4-P) and the other was shale-derived (JP4-S).

EXPOSURE TO TOXICANTS

Exposure of Plants to HF

Plants were grown to the required age then transported 0.5 mile to the fumigation facilities on the morning of the exposure.

The generator oven was heated to ca. 100°C. Power was supplied to the heat cable, chamber paddles, exhaust fan, and carrier air flow. HF solution was drawn into the syringe. The syringe pump was started after the oven had been on 45 minutes. Two chamber air samples were made before actual fumigations began. Plants in pots or flats were placed in the chambers and fumigation commenced. Five air samples were withdrawn during fumigation. Plants were removed after 20 minutes and HF solution was changed if another fumigation at a different gas concentration was scheduled. Plants were transported back to the glasshouse where they were graded 24 hours after exposure. Syringes and generator lines were flushed with distilled water for 1 hour after completing the fumigation. Chambers were exhausted for 0.5 hours.

Seeds were exposed to HF gas on wet filter paper in open Petri dishes by placing the dishes in the exposure chambers in the same manner as for plants. In most cases the seeds were transferred to another set of Petri dishes with unexposed filter paper disks since the disks adsorbed significant amounts of HF during exposures. After exposure the dishes were covered and returned to the laboratory for incubation. Germination rate and seedling lengths were recorded at a set time after treatment.

Jet Fuel Exposures

For spray or mist applications of jet fuel, plants were put into the Lexan chamber and the spinning-disk applicator was started. Fuel was slowly introduced into the applicator. Treatment was complete within five minutes. Plants were removed and left outside the glasshouse for 6 to 12 hours until fumes dissipated. An exhaust fan evacuated the chamber. Plants were graded 48 to 72 hours after treatment.

Plants exposed to fuel vapor were sealed in the Lexan chamber. Fuel was applied to a suspended sheet of absorbent paper towel using a pipette inserted through a hole in the chamber top. The plants were removed after 20 minutes and graded for injury after 48 to 72 hours.

TABLE 1
PLANT SPECIES USED IN HF AND FUEL EXPERIMENTS

Common name	Latin Name	Variety or Cultivar
Alfalfa	*Medicago sativa* L.	Cu Fl01, Bonanza
Barley	*Hordeum vulgare* L.	CM67
Bean	*Phaseolus vulgaris* L.	Pinto U.I. III
Carrot	*Daucus carota* L. subsp. *sativus* [Huffm.] Arcang.	Red-cored Chantenay
Corn	*Zea mays* L. Subsp. *mays*	HX980, Golden Cross and Bantam
Cotton	*Gossypium hirsutum* L.	SJ2
Lettuce	*Lactuca sativa* L.	Black Seeded Simpson
Pea	*Pisum sativum* L.	Green Arrow
Radish	*Raphanus sativus* L.	Cherry Belle
Sorghum	*Sorghum bicolor* [L.] Moench	Sweet Sorghum
Squash	*Cucurbita moschata* Duchesne	Early Prolific
Sudan-grass	*Sorghum sudanense* [Piper] Stapf	Piper
Sun-flower	*Helianthus annuus* L.	Teddy Bear
Tomato	*Lycopersicon esculentum* Mill.	Ace 55 VF
Wheat	*Triticum aestivum* L.	Yecora Rato
Zinnia	*Zinnia elegans* Jacq.	Scarlet Queen

TABLE 2
COMPOSITION OF UC SOIL MIX II

Components	Amounts
Soil (Oakley sand)	0.40 m^3 (14 ft^3)
Canadian peat moss	0.20 m^3 (7 ft^3)
Redwood shavings or fir bark	0.20 m^3 (7 ft^3)
Single super phosphate	1.13 kg (2.5 lbs)
KNO_3	0.11 kg (4 oz)
K_2SO_4	0.11 kg (4 oz)
Dolomite limestone	1.70 kg (3.75 lbs)
Oyster shell lime	0.68 kg (1.5 lb)
Micronutrients	
Cu	30 ppm (dry basis)
Zn	10 ppm (dry basis)
Mn	15 ppm (dry basis)
Fe	15 ppm (dry basis)

Drenches of soil with jet fuel were accomplished by applying fuel to the soil surface or mixing it with soil and planting seeds as described earlier. Flats or pots of treated soil were returned to the glasshouse for seed incubation 6 to 12 hours after treatment. After a certain period seedling emergence was recorded and, in some cases, seedlings were washed free of soil and shoot and root lengths were measured.

Fuel movement in soil was tested by observing inhibition of seedling emergence horizontally from point of application. Vertical movement was estimated by layering fuel on a column of soil and allowing it to percolate downward. The soil column was sectioned and each section tested for any effect on seed germination and seedling development. The equipment and technique for this work is described in detail below.

MEASUREMENTS

Injury

Plants were examined for injury due to exposure to HF gas or jet fuel 24 to 72 hours after treatments, a period which allowed for recovery from transient wilting and for development of bronzing, glazing, chlorosis, and necrosis. Each leaf of the treated plant was graded for percent leaf area injured using a 1-12 rating scale (Horsfall and Barratt, 1945; Horsfall and Cowling, 1978). The scale compensated for the tendency of the eye to attach greater importance to small differences in injury at damage level extremes (Table 3). During the HF test, six plant species were exposed on a regular basis and graded. It was convenient to develop a set of keys to which actual leaves were compared. These keys are illustrated in Appendix A.

Seed Tests

In tests where soil was contaminated by jet fuel or where seeds were exposed to HF gas, injury was recorded as numbers of germinated seeds or emerged seedlings. In some cases, seedling lengths were also measured. When a treatment reduced germination, fewer seedlings could be measured and length analysis was more difficult.

Pollutant Measurements

Hydrogen fluoride gas concentration was measured by bubbling 15 liters of contaminated atmosphere through aqueous 0.01 N sodium hydroxide (NaOH) in a bubbler constructed from a modified plastic graduated cylinder (Intersociety Committee, 1969). The resulting solution was mixed with total ionic strength adjusting buffer (TISAB) and tested for fluoride ion with an F-specific electrode on an Orion model 901 ionanalyzer (Orion Research, 1977). Gas concentration was expressed as mg HF m^{-3}. Five 15-liter air samples were drawn during each 20-minute fumigation.

Environmental levels of jet fuel were difficult to estimate since fuel is composed of many different compounds (Ivens, 1952). Amount of fuel

TABLE 3
SCALE FOR GRADING FOLIAR NECROTIC INJURY

Grade[1]	Percent necrosis (%)
1	0 (no visible injury)
2	3
3	3-6
4	6-12
5	12-25
6	25-50
7	50-75
8	75-87
9	87-94
10	94-97
11	97
12	100 (death of leaf)

[1]Numerical grade is arbitrary; scale is based on Horsfall and Barratt (1945)

injected into the spinning-disk applicator (in ml) was used to quantify spray work. Volume (in ml) of fuel applied to the absorbent paper was used as a measure of concentration for vapor studies. Fuel drench concentrations were measured by calculating the applied fuel per surface area or soil volume (ml fuel cm^{-2}, ml g^{-1} or ml m^{-3}).

Environmental Parameters

Temperature, relative humidity, and light intensity were measured as previously described (Granett and Taylor 1981).

GENERATION AND PHYTOTOXICITY OF HF GAS

HF GENERATING SYSTEM

System Development

The HF gas generation system was developed following a system described by McLean et al. (1968).

Teflon rods were machined into two syringes (Figure 6), each with a barrel length of 125 mm, an outside diameter of 30 mm, and an inside dia-

meter of 23 mm. Syringe capacity was 40 ml. Neoprene o-rings set in grooves cut in the plunger marked 5-ml intervals. A thicker o-ring near the plunger tip effectively sealed the pieces and prevented HF solution from flowing out the back of the syringe. A Teflon tube with flange fittings connected the front of the syringe to a three-way miniature Teflon-and-stainless-steel valve. Turning the valve in one direction connected the syringe to 1.6-mm tubing entering the generating oven whereas in the other direction HF solution could be drawn into the syringe without removing it from the pump. Two valves mounted on one bracket allowed both syringes to operate on a single syringe pump.

The Teflon syringes were calibrated using two pumps, and actual delivery was calculated for different flow rates (Table 4). Differences between the two pumps or between the two syringes were insignificant.

The air system (Figure 8) operated at ca. 12 liters min^{-1} per chamber. Reserve tank pressure was maintained at 15 psig with a single cylinder 0.25-hp air pump; a pancake regulator maintained output pressure at 3 psig. A heatless air dryer removed moisture from the compressed air by passing it through a desiccant.

The air system was checked for leaks with CO gas using a Beckman infrared analyzer. No CO was detected outside the oven or chambers except at the exhaust outlet. Copper tubing and Swagelok fittings connecting all parts were tightened or replaced inside the system until no gas leaks were found.

CO gas and the Beckman analyzer were also used to determine the air exchange rate through the two chambers (Table 5).

Safety Checks

Safety checks were conducted to assure that no HF gas was escaping during generation. When 1.25 mg HF m^{-3} was generated in one chamber, negligible HF was detected in the exhaust (after passing through the limestone filter), in the control chamber, in the glasshouse, and at the air intake of the fumigation chamber (Table 6). Samples were collected with bubblers.

Generation Calibration

Gaseous HF measurements obtained using bubblers were highly correlated with percent HF injected. Regression analysis indicated good fit ($r = 0.987$) to a linear relationship between injected and gaseous HF, making predictable the concentration expected in the chamber (Figure 11). HF concentration for a 20-minute fumigation was the mean HF of five chamber atmosphere samples collected during the exposure period. Gas concentrations measured in the north and south chambers when the same solution was injected through the generator did not differ significantly.

TABLE 4
CALIBRATION OF SYRINGES ON SYRINGE PUMPS

Pump flow setting (ml min^{-1})	Actual delivery rate (ml min^{-1})
0.2	0.18 ± 0.01
0.3	0.27 ± 0.01
0.4	0.36 ± 0.02

TABLE 5
CALCULATIONS OF CHAMBER AIR CHANGE RATE

Chamber	Volume (m^3)	CO rate[1] (ml min^{-1})	CO concn (ppm)	Air changes per minute
South	1.07	385.7	305[2]	1.170
North	1.06	386.8	275	1.317

[1]Calculated by timing bubble movement in flowmeter
[2]Chamber concentration from 99.3% CO tank

TABLE 6
HF GAS (mg m^{-3}) DETECTED DURING FUMIGATION

Sample site	Standard used	
	1 ppm HF	Distilled water
Exhaust fan	0.026	0.002
Control chamber	0.026	0.005
Glasshouse	0.020	0.004
Fumigation chamber inlet	0.017	0.004
Fumigation chamber	1.25	NS[1]

[1]NS = not sampled

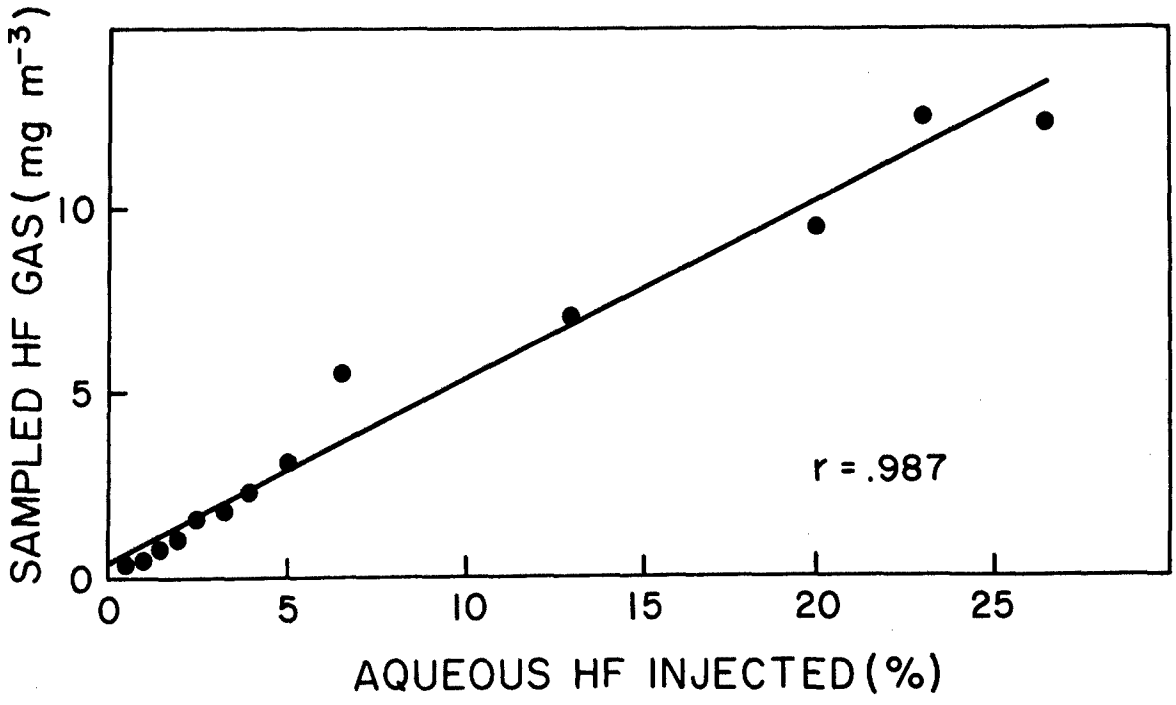


Figure 11. Concentration of chamber HF gas obtained by injecting aqueous hydrofluoric acid solutions into generator.

Bubbler Efficiency

The efficiency of bubblers constructed of modified graduated cylinders was tested by connecting two such units in series and analyzing the solutions in both (Table 7). In one test, no HF was detected in the second sampling device when both bubblers sampled 20 liters of chamber atmosphere. In another test, five separate 15-liter samples of chamber atmosphere were drawn through the first bubbler, while the solution in the second bubbler was removed only after all 75 liters were bubbled through it. Four 20-minute fumigations were analyzed. Again, negligible amounts of gas were detected in the second bubbler. Plants present in the chamber during tests 3, 4, and 5 may have absorbed some pollutant and so reduced the amount of HF trapped. It was concluded that a single bubbler trapped all HF gas passing through it.

Measuring HF with Filter Paper

Another method for estimating the concentration of HF in the exposure chamber was to collect it onto filter paper discs (Huygen, 1963). Two ml of 0.01 N NaOH were applied to each of four 9-cm filter disks one day before exposure. The dried disks were placed on the chamber floor during a 20-minute exposure and were then transferred to plastic vials containing 20 ml 0.01 N NaOH. After 15 minutes the paper was removed and the eluate was

TABLE 7
SAMPLING CHAMBER HF CONCENTRATION (mg m^{-3}) USING TWO BUBBLERS IN SERIES

Test	First bubbler		Second bubbler	
	Sample (liters)	HF conc (mg m^{-3})	Sample (liters)	HF conc (mg m^{-3})
1	20	26.6 ± 3.5[1]	20	0.04 ± 0.02[2]
2	15	12.0 ± 1.1[3]	75	0.20[4]
3	15	10.5 ± 0.6	75	0.04
4	15	9.7 ± 1.1	75	0.04
5	15	9.7 ± 0.6	75	0.04

[1]Mean and standard deviation of four bubbler samples
[2]20-liter samples in second bubbler taken at same time as first bubbler
[3]In tests 2-5, listed concentration is mean and standard deviation of five separate 15-liter samples drawn during a single 20-minute exposure period
[4]Concentration was based on one 75-liter sample drawn during same 20-minute period as all five samples for first bubbler

measured with the F-specific electrode. Unexposed control paper had 0.3 ppm F. The meancontent of four disks exposed for 20 minutes to 12.0 ± 1.1 mg HF m^{-3} was 25.6 ± 1.9 ppm F. Since the paper collected HF by deposition, direct calculations of gas concentrations were not appropriate.

PHYTOTOXICITY OF GASEOUS HF

Age and Injury

Tests were conducted to determine system performance and the effect of age on bean injury. HF gas was generated at 3.9 ± 0.5 and 8.5 ± 1.6 mg HF m^{-3} in the two chambers, when 6.5 and 13% aqueous HF solutions were injected, respectively (Table 8). Samples were drawn at five-minute intervals (Figure 12 and 13). Gas concentrations changed rapidly when the syringe pump was activated or stopped. Placing plants into the exposure chambers decreased measurable HF gas concentration about 0.5 mg m^{-3} for 10 to 15 minutes.

Bean plants of different ages were exposed for 20 minutes during the 100 minute gas generation trials. Bleaching was observed 30 minutes after plants were removed from the chamber and after 24 hours tissue had become necrotic. Bronzing was observed on the abaxial sides of leaves that were not completely necrotic. Injury was estimated for each leaf (Table 9). Larger gas concentrations produced more injury. Injury decreased with age

TABLE 8
CHAMBER HF CONCENTRATION (mg m^{-3}) FOR TWO AQUEOUS HF SOLUTIONS

6.5% HF			13% HF		
Trial	No. samples drawn	Chamber concn ($mg\ m^{-3}$)	Trial	No. samples drawn	Chamber concn ($mg\ m^{-3}$)
1	12	3.75 ± 0.43[1]	1	22	8.49 ± 0.71
2	15	3.70 ± 0.36	2	14	8.19 ± 0.65
3	10	4.42 ± 0.42	3	15	8.93 ± 2.89
	Average	3.91 ± 0.50		Average	8.54 ± 1.65

[1]Chamber concentration in mg HF m^{-3}; mean and standard deviation of 10 to 22 samples

TABLE 9
LEAF INJURY ON BEAN PLANTS EXPOSED TO HF GAS FOR 20 MINUTES

Plant age (days)	HF concn ($mg\ m^{-3}$)	Injury[1]	
		Primary leaves	Secondary leaves
14	3.5	10.2 ± 1.0	-
18	4.5	11.1 ± 0.8	2.4 ± 0.8
20	3.3	10.6 ± 1.4	2.8 ± 1.6
23	3.5	3.1 ± 1.0	5.9 ± 1.0
25	4.5	8.0 ± 2.1	8.8 ± 1.0
28	3.3	5.8 ± 2.6	9.1 ± 2.1

[1]Necrosis estimated on 1-12 scale; each value represents data from 10 bean plants

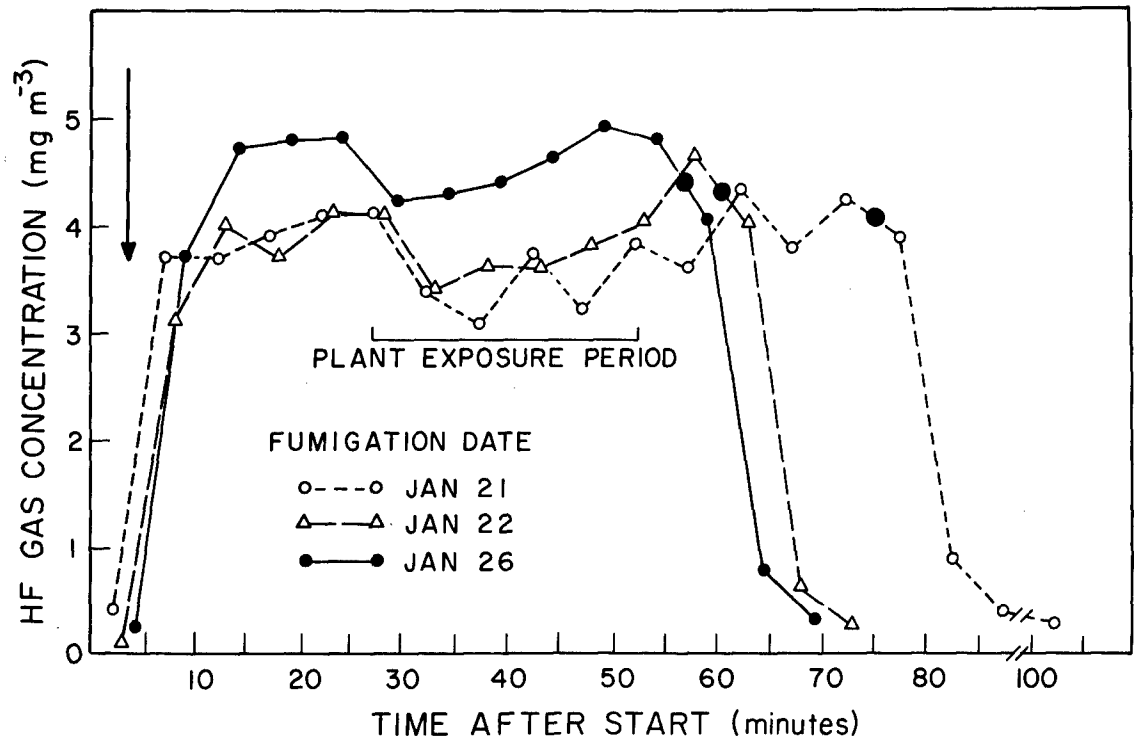


Figure 12. Chamber HF gas concentration for three trials using 6.5% hydrofluoric acid. Syringe pump on at arrow; pump off at large solid circles.

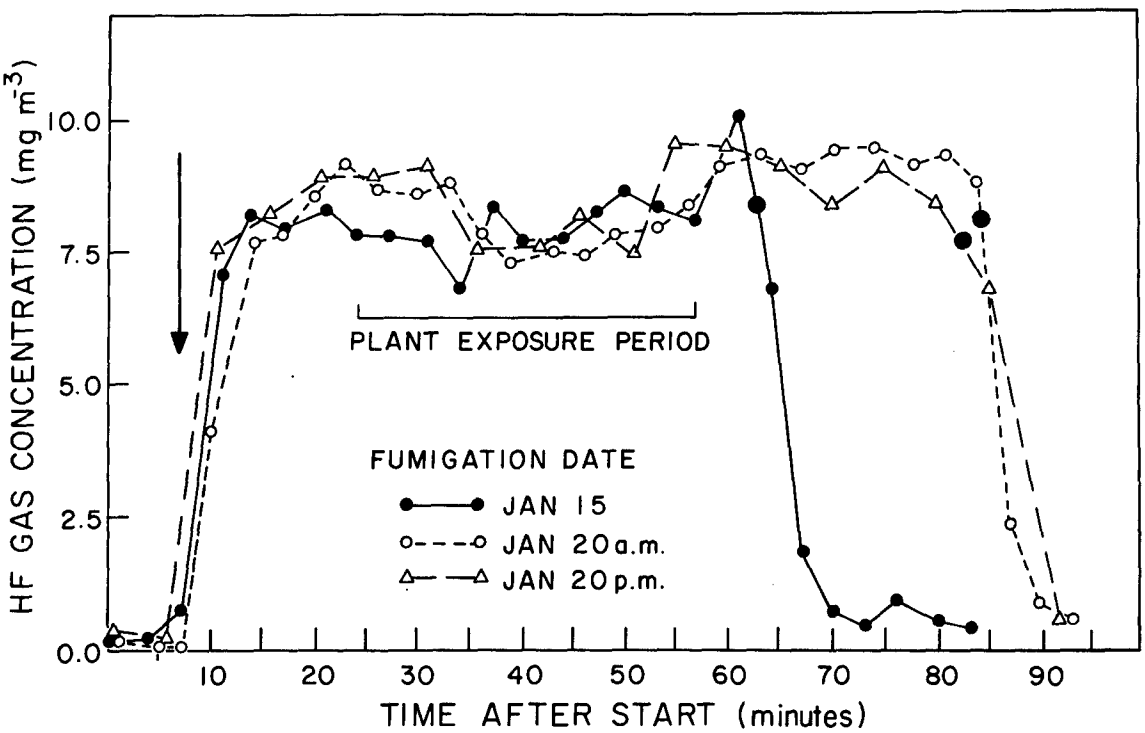


Figure 13. Chamber HF gas concentration for three trials using 13% hydrofluoric acid. See caption for Figure 12.

for primary leaves, probably due to onset of senescence. Injury on secondary leaves increased as the leaf aged.

Dose Response of Six Species

In a six-week experiment, groups of bean, barley, lettuce, radish, tomato, and zinnia seedlings were exposed to HF gas for 20 minutes. Exposures occurred twice a week at 11:00 AM, 11:30 AM, and 12:00 noon. Six exposures (three times x two chambers), one for each species, took place on each day. HF gas for all six exposures was generated using the same HF solution; sampled HF chamber concentration varied slightly. Concentrations for the series, ranging from 0.4 to 12.6 mg HF m^{-3}, were generated using 0.5 to 26% aqueous HF acid. Chamber concentrations were verified by drawing five 15-liter bubbler samples during the 20-minute exposure period. The time of day a particular species was fumigated was randomly assigned but age of plant at time of exposure was fixed (Table 10). The order of the concentrations during the six-week experiment was also randomized to minimize the effect of fluctuating temperature (Table 11). Environmental conditions were monitored but could not be controlled and temperature extremes occurred (Table 12). During each fumigation three fiber 10 x 13-inch flats containing 20 plants each were exposed. Three leaves from each of 15 plants per flat were graded on the day after exposure using the pictorial keys (Appendix A).

Plant injury was obtained even at the lowest levels of HF gas. With larger gas concentrations, injury in the form of bleaching and wilting was often observed while plants were still in the fumigation chambers. In time, the bleached areas became necrotic. Large doses created necrotic leaf margins and veins, whereas at small doses most injury was intercostal (Figure 14). With even smaller doses, necrosis was absent and glazing was observed on abaxial surfaces. Plants were not killed at 12.6 mg HF m^{-3}, the largest dose used.

TABLE 10
TYPICAL RANDOMIZED ASSIGNMENT OF FUMIGATIONS

Chamber	Time of day		
	11 AM	11:30 AM	12 PM
North	Radish (21)[1]	Bean (14)	Lettuce (28)
South	Tomato (28)	Zinnia (28)	Barley (17)

[1]Plant age ( ), in days, at time of fumigation

TABLE 11
CONDITIONS EXISTING DURING HF FUMIGATIONS

Date (1981)	Acid injected (%)	HF concentration[1] (mg m^{-3})	Temperature (C^o)	Relative humidity (%)	Light[4] ($x10^5$)[3]
4/28	4.0	2.3 ± 0.3	45	-[2]	-
4/30	1.5	0.8 ± 0.1	45	-	2.6
5/5	3.3	1.8 ± 0.3	32	-	2.4
5/7	2.5	1.6 ± 0.1	36	46	2.8
5/12	6.5	5.6 ± 0.5	39	-	2.7
5/14	2.0	1.0 ± 0.2	27	52	0.7
5/19	1.0	0.4 ± 0.0	26	51	0.5
5/21	20.0	9.5 ± 0.8	38	37	3.8
5/26	0.5	0.3 ± 0.1	34	58	2.6
5/28	13.0	7.1 ± 1.3	40	48	3.2
6/2	26.0	12.6 ± 1.6	28	64	0.9
6/4	5.0	3.0 ± 0.5	48	21	3.5
6/9	23.0	12.3 ± 1.9	44	50	3.2

[1]Mean and standard deviation of six fumigations
[2]Data not available
[3]Light intensity, in ergs cm^{-2} s^{-1}

The response of each plant to gaseous HF was plotted as data points (Figure 15). In most cases foliar injury increased rapidly with increasing HF up to about 5 mg HF m^{-3} while doses greater than 5 mg m^{-3} produced damage approaching the maximum of 12. Regression curves were fitted to logarithmically transformed data, and may be interpreted as the predicted response of each plant species to a given dose of HF. Variations of specific data points from the calculated lines may reflect differences in individuals and reactions to changes in the environment; correlations of injury with light intensity were significant for barley and pinto bean (Table 12). Correlations of injury with temperature or relative humidity were not significant.

The dose of HF required for 50% leaf injury (Table 13) was derived from the curves. Ratings of species sensitivity (sensitive, S; intermediate, I; or resistant, R) could be assigned on the basis of these 50% doses. Thus zinnia seedlings at 6.75 mg m^{-3} were most resistant and radish at 1.8 mg m^{-3} were most sensitive of the species tested. A review compiled by the National Academy of Sciences (1971) listed the susceptibility of various plant species to long-term, short doses of HF gas. Those results can be compared to ours (Table 14), keeping in mind that our work was with short-term, large doses. We found tomato, pinto bean, and radish more susceptible under our conditions, and bar-

Figure 14. Lettuce (top row) and radish leaves exposed to HF gas. First column illustrates glazing while other leaves suffer bifacial necrosis.

ley more resistant. It appears that prediction of injury in a particular plant may be feasible only when doses are comparable.

Hydrogen chloride gas (HCl) is about one-tenth as toxic as HF (National Academy of Sciences, 1971; Guderian, 1977). Either gas can be released by rocket engines depending on solid fuel composition. Sensitivities of six species to HCl or HF gas at high concentrations were compared (Table 15). Differences in sensitivity of zinnia may be due to differences in varieties used. Tomato and barley seedlings were more sensitive to HF than to HCl.

EXPOSING SEEDS TO GASEOUS HF

Post-exposure Rinsing

Initial seed experiments tested the general reaction of seeds to gas. Groups of 25 tomato seeds were exposed for 20 minutes to 4.5 mg HF m^{-3}, on moist or dry filter paper disks. Immediately after exposure, groups of seeds were rinsed three times in distilled-deionized water (DDW) or remained unrinsed. All seeds were transferred to unexposed filter paper disks in clean plastic Petri dishes. Two ml of DDW was added before the dishes were covered and sealed with Parafilm.

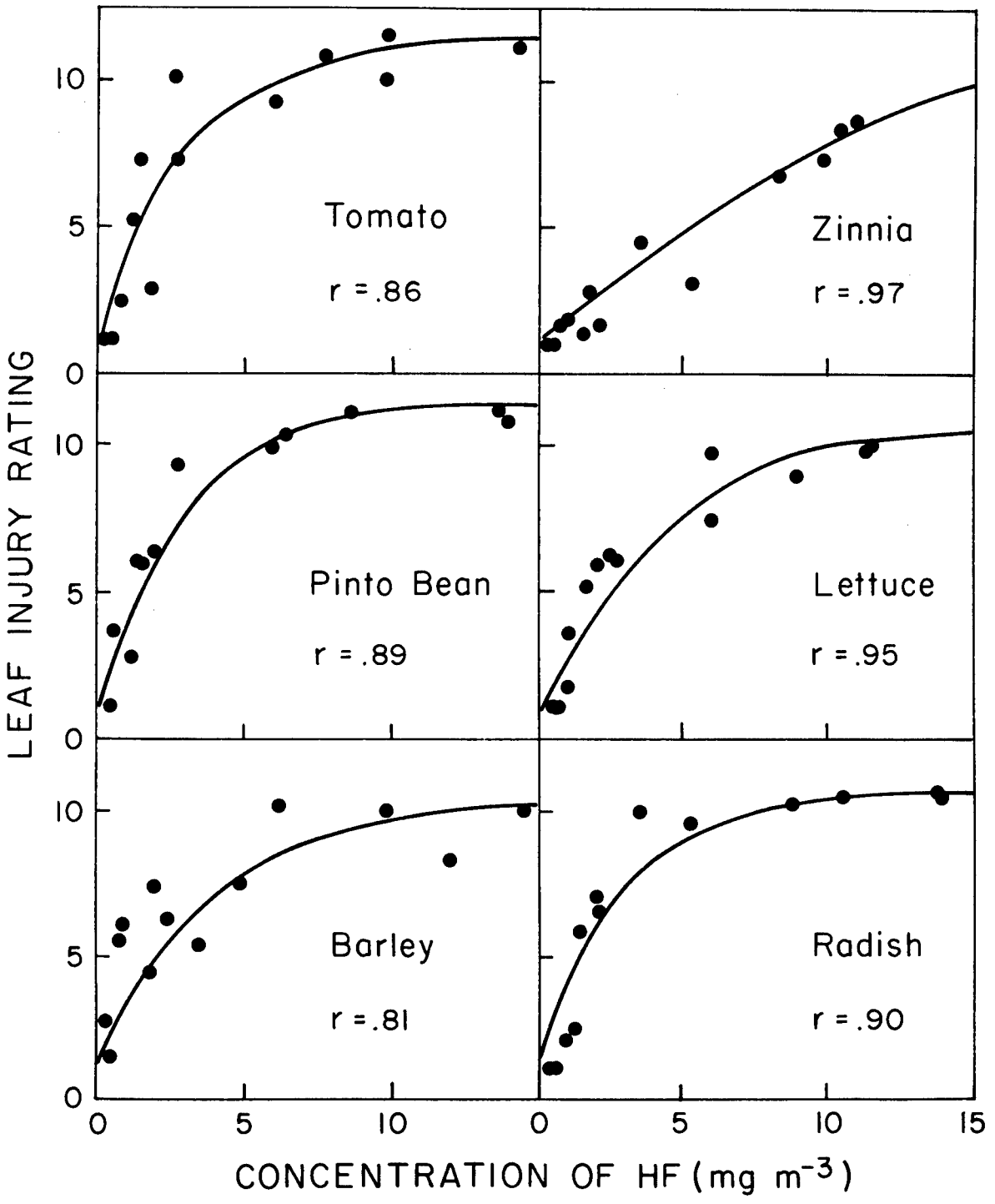


Figure 15. Response of six species to 20-minute exposures to HF gas; r value is regression coefficient for curve fitted to data points

TABLE 12
CORRELATION OF INJURY WITH HF GAS CONCENTRATIONS AND ENVIRONMENTAL FACTORS

Species	Gas concentration	Light intensity	Temperature	Relative humidity
Barley	0.7738 *[1] p = 0.001	0.4733 * p = 0.051	0.3988 p = 0.089	-0.0386 p = 0.450
Lettuce	0.8899 * p = 0.0001	0.3401 p = 0.128	0.2745 p = 0.182	-0.0243 p = 0.469
Pinto	0.7989 * p = 0.001	0.5196 * p = 0.034	0.4170 p = 0.078	-0.0804 p = 0.397
Radish	0.8032 * p = 0.0001	0.2725 p = 0.184	0.2976 p = 0.162	-0.0532 p = 0.431
Tomato	0.7922 * p = 0.001	0.2919 p = 0.167	0.3910 p = 0.093	0.1622 p = 0.298
Zinnia	0.9733 * p = 0.0001	0.3034 p = 0.157	0.2435 p = 0.211	0.3679 p = 0.108

[1]Correlation coeffient, r; p = probability; * indicates significance at 5% level

TABLE 13
DOSE OF HF NEEDED FOR 50% INJURY TO LEAVES IN 20-MINUTE EXPOSURE

Species	HF Concentration ($mg\ m^{-3}$)
Radish	1.8
Tomato	2.0
Bean	2.0
Barley	3.0
Lettuce	3.5
Zinnia	6.8

TABLE 14
SUSCEPTIBILITY OF PLANT SPECIES TO HF GAS

Species	Ratings[1]	
	UCR[2]	NAS[3]
Radish	S	R
Tomato	S	I to R
Bean	S	I
Barley	I	S
Lettuce	I	I to R
Zinnia	R	I to R

[1]S = sensitive to HF injury; I = intermediate; R = resistant
[2]University of California, our present finding determined by relative ranking of 50% injury dose
[3]National Academy of Sciences (1971)

TABLE 15
COMPARISON OF RELATIVE SENSITIVITIES OF SELECTED PLANTS TO GASEOUS HCl OR HF

Species	Cultivar	Toxic gas			
		HCl[1]		HF	
		Dose[2]	Rank[3]	Dose[2]	Rank[3]
Bean	Pinto	15	VS	1.8	S
Radish	Comet	25	S	2.0	S
Zinnia	Cherry Gem	25	S	-	-
Tomato	Ace	50	I-R	2.0	S
Barley	CM 67	75	R	3.0	I
Zinnia	Scarlet Queen	-	-	6.8	R

[1]HCl data from Granett and Taylor, 1980a
[2]Dose is HCl or HF concentration in mg m^{-3} needed to cause 50% leaf injury in 20 minutes
[3]VS = very sensitive; see Table 14 notes

After seven days, the germinated seeds were counted (Table 16) and seedling shoots and roots were measured (Table 17). Seeds exposed in a control chamber and controls not placed in the exposure chamber had 96-100% germination and developed seedlings with apparently normal lengths. Seeds exposed to gaseous HF had greatly reduced germination and surviving seedlings developed

TABLE 16
GERMINATION OF TOMATO SEEDS EXPOSED TO HF GAS FOR 20 MINUTES AND INCUBATED IN PETRI DISHES

Treatment before exposure	Treatment after exposure	Exposure treatment		
		6.5 mg HF m^{-3}	0 mg HF m^{-3}	Unexposed controls[1]
Dry	None	9[2]	100	100
	Rinse	52	100	96
Moist	None	0	100	100
	Rinse	0	100	100

[1]Unexposed seeds not placed in fumigation chamber
[2]Percent germination of 15 tomato seeds; mean of three cups of seeds

TABLE 17
LENGTH OF TOMATO SEEDLINGS INCUBATED FOR SEVEN DAYS IN PETRI DISHES AFTER EXPOSURE TO HF GAS

Treatment before exposure	Treatment after exposure	Exposure Treatment					
		4.5 mg HF m^{-3}		0 mg HF m^{-3}		Unexposed controls[1]	
		Shoots	Roots	Shoots	Roots	Shoots	Roots
Dry	None	2±3[2]	10±11	24±7	46±8	26±6	54±12
	Rinse	12±9	30±16	26±7	60±15	21±7	52±20
Moist	None	0	0	29±9	53±20	20±5	39±8
	Rinse	0	0	25±6	56±15	19±6	59±17

[1]See Table 16
[2]Mean lengths, in cm, of those seeds that have germinated, 45 seeds maximum

poorly. Seeds that were wet when exposed to HF did not germinate. When dry seeds were rinsed immediately after HF exposure, both germination and subsequent seedling lengths increased compared to unrinsed seeds.

Incubating Seeds in Soil Exposed to HF Gas

Tomato seeds exposed to HF gas did not develop normally when incubated in Petri dishes, an artificial environment in which HF effects on seeds were not buffered. In the next experiment, seeds were sown in soil which was then exposed to the toxicant.

Soil in 350-ml Styrofoam cups was watered three hours before fumigation. Tomato seeds were planted 5 mm deep, 15 per pot, 30 minutes before or 30 after exposing soil to 3.0 mg HF m^{-3} for 20 minutes. Controls, all planted before treatments, were placed in a chamber with 0 mg HF m^{-3} for the same period. There were three cups per treatment.

Germination was recorded daily beginning seven days after fumigation and continuing for 11 days (Figure 16). Final percent emergence was 78 ± 4% for seeds planted before soil exposure, and 90 ± 14% for seeds on soil unexposed to HF gas. Seedling total lengths and dry weight were measured 25 days after fumigation (Table 18). Although germination rate and lengths were greatest for control seeds and smallest for seeds exposed to HF in the soil, these differences were not statistically significant at 5% level for this gas concentration.

Incubating HF-Exposed Seeds in Soil

In the next experiment, seeds were exposed to HF gas directly and then planted. Groups of 15 tomato seeds were rinsed in DDW and exposed to 0 or 3 mg HF m^{-3} on filter paper disks moistened with 1 ml DDW or were exposed on dry filter paper. Some groups of seeds were rinsed in DDW following exposure, then all seeds were sown in 350-ml cups of soil. The perforated cups were set on trays of water for the first week of seed incubation and were watered from above thereafter. Treatments were replicated three times. Percent emergence, seedling lengths, and dry weights were measured 25 days after sowing. The means of different treatments were summarized (Table 19). Analyses of variance indicated that no significant differences at the 5% level existed between the means for the several treatment factors.

Four Species of Seeds Exposed to HF Gas

Radish, tomato, lettuce, and barley seeds, wetted with DDW and placed on moist filter paper, were exposed to 10 mg HF m^{-3} for 20 minutes. After exposure, seeds were transferred without rinsing to Petri dishes lined with unexposed filter paper disks (one disk of 10 seeds per species) or were transferred to cups of soil (three cups of 10 seeds each per species) and covered to an appropriate depth with soil. Unexposed seeds served as controls (checks). Seeds in Petri dishes were incubated in the laboratory in the dark and root and shoot lengths were measured one week after treatment (Table 20). Seeds sown in cups were maintained in the glasshouse and watered as needed. Shoot lengths

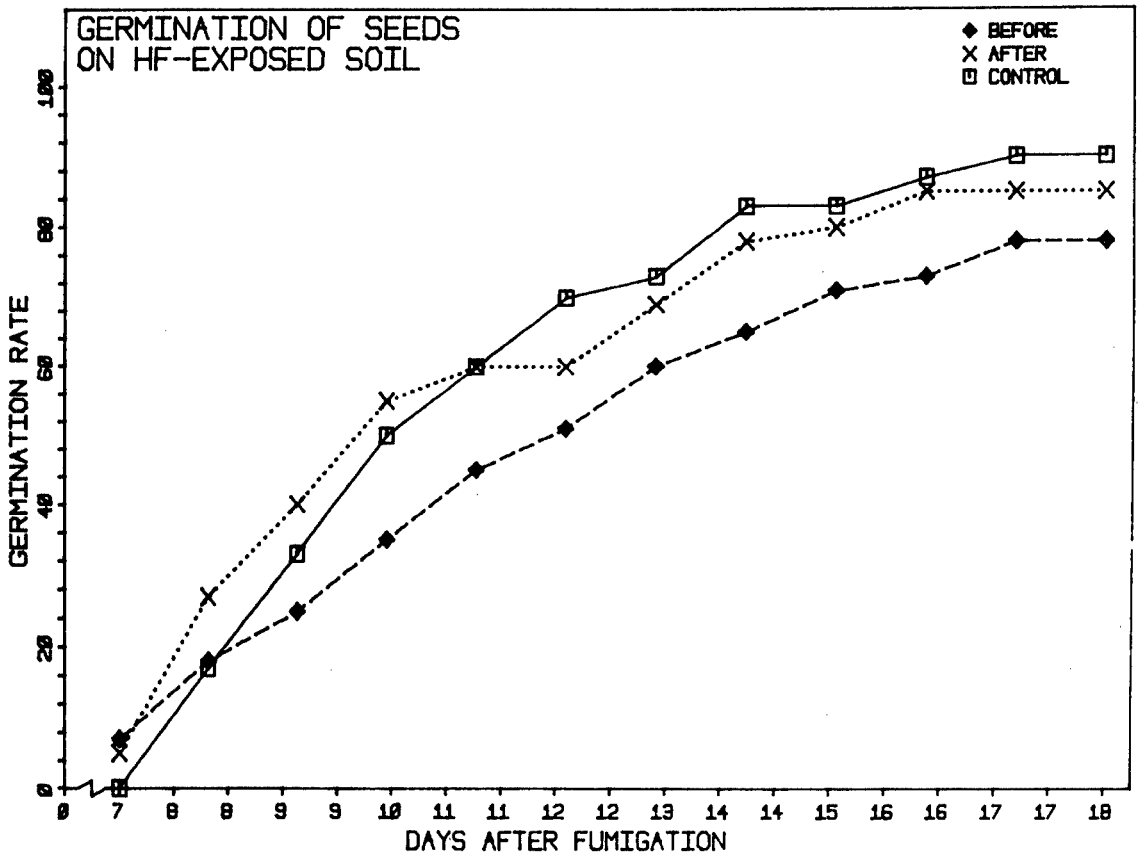


Figure 16. Germination of tomato seeds on soil exposed for 20 minutes to HF gas before or after seeds were planted.

TABLE 18
DEVELOPMENT OF TOMATO SEEDS IN SOIL AFTER EXPOSURE TO 3.0 mg m^{-3} HF GAS FOR 20 MINUTES

Treatment	Emergence (%)	Lengths (mm)	Dry weights (mg)
Soil exposed <u>after</u> seeds planted	78 ± 4[1]	105 ± 42[2]	74 ± 58
Soil exposed <u>before</u> seeds planted	84 ± 10	129 ± 39	62 ± 64
Unexposed control of soil not exposed to HF	90 ± 14	138 ± 128	61 ± 41

[1]Emergence of seedlings; mean and standard deviation of three pots per treatment
[2]Lengths and dry weights of seedlings; mean and standard deviation of up to 45 seedlings

TABLE 19
DEVELOPMENT OF WET TOMATO SEEDS EXPOSED TO HF GAS THEN RINSED AND GROWN IN SOIL

Treatment	No. means averaged	Emergence (%)	Length (mm)	Dry weight (mg)
Wet	4	76 ± 13[1]	112 ± 43	66 ± 57
Dry	4	48 ± 22	106 ± 39	61 ± 52
Rinsed	4	62 ± 17	110 ± 43	59 ± 56
Unrinsed	4	70 ± 19	109 ± 40	68 ± 54
Exposed	4	67 ± 16	106 ± 43	57 ± 50
Unexposed	4	65 ± 23	115 ± 38	76 ± 72
Exposed wet	2	72 ± 15	105 ± 44	56 ± 48
Exposed dry	2	48 ± 26	104 ± 42	58 ± 54
Unexposed wet	2	82 ± 8	117 ± 42	81 ± 68
Unexposed dry	2	48 ± 20	110 ± 30	67 ± 50
Exposed & rinsed	2	67 ± 8	108 ± 43	52 ± 44
Exposed & unrinsed	2	67 ± 22	116 ± 33	62 ± 55
Unexposed & rinsed	2	55 ± 26	113 ± 44	72 ± 73
Unexposed & unrinsed	2	75 ± 17	105 ± 43	78 ± 51
Exposed wet & rinsed	1	72 ± 20	109 ± 42	47 ± 40
Exposed wet & unrinsed	1	82 ± 15	108 ± 42	64 ± 52
Exposed dry & rinsed	1	71 ± 8	106 ± 44	57 ± 48
Exposed dry & unrinsed	1	51 ± 14	101 ± 40	59 ± 60
Unexposed wet & rinsed	1	77 ± 5	115 ± 49	77 ± 79
Unexposed wet & unrinsed	1	87 ± 9	119 ± 35	84 ± 55
Unexposed dry & rinsed	1	33 ± 9	110 ± 29	62 ± 58
Unexposed dry & unrinsed	1	63 ± 14	111 ± 31	70 ± 45

[1]Mean and standard deviation for emergence, length, and dry weight

were recorded two weeks after treatment (Table 21). Tomato seedlings were measured again four weeks after exposure.

Of the seeds incubated in the laboratory, lettuce was the only species where growth was significantly inhibited (at 5% level), although the germination of tomato seeds was decreased by half.

Germination of tomato, barley, and lettuce was decreased for exposed seeds sown in soil, but of these only tomato seedling length was significantly reduced (at 1% level) compared to unexposed controls.

Sensitivity of Seven Species of Seeds to HF Gas

Seeds of barley, bean, lettuce, radish, squash, tomato, and zinnia were exposed to 5.3 ± 0.5 mg HF m^{-3} for 20 minutes. Seed types were chosen for rapidity of germination, previous use as test species in other air pollution experiments (Heck et al., 1979; Hicks, 1968) and as representatives of a range of botanical and horticultural types from monocots to dicots and from field crops and ornamentals to garden varieties. The seeds, either soaked for two hours in DDW or wetted just prior to HF exposure, were placed on moist filter paper disks during fumigation. Control seeds were wetted and placed in Petri dishes which were left open in the glasshouse during the 20-minute fumigation period.

After exposure, seeds were transferred to unexposed filter paper disks and incubated in the dark or were placed on the surface of moist soil in cups, covered with soil, and incubated in the glasshouse. Seedlings in Petri dishes were counted and total lengths were measured eight days after treatments (Table 22). Roots of seedlings grown in soil were washed three weeks after treatment and total lengths were measured (Table 23). All seeds in these tests were affected by HF gas (Table 22). Squash appeared somewhat resistant to the treatment and the few barley seeds that germinated after treatment had limited growth. Beans did not develop normally in Petri dishes even without gas fumigations.

Seeds planted in soil grew better than those in Petri dishes (Table 23). Only lettuce and tomato seeds seemed affected by HF gas when lengths were compared. An overall reduction in emergence of the seeds soaked two hours before exposure was evident with all species. Only treated tomato seedling lengths were significantly reduced compared to controls and the reduction was greater with the soaked treatment than with the briefly wetted seeds.

Considering all the seed tests, exposing seeds to HF gas reduced germination or emergence and subsequent seedling lengths only under certain conditions. Concentration had to be relatively large, and seeds became more sensitive when soaked or allowed to imbibe water prior to fumigation. Dry or briefly wetted seeds were less sensitive than soaked ones. Seeds confined to Petri dishes were more susceptible to HF injury than those sown in soil. Soil may buffer or adsorb and neutralize HF acid adsorbed to the seed. Of the species tested, tomato seeds were consistently sensitive to HF gas; squash and radish may be particularly resistant.

TABLE 20
GERMINATION AND SEEDLING LENGTH OF FOUR SPECIES OF SEEDS EXPOSED TO 10 mg HF m^{-3} AND INCUBATED IN LABORATORY

Measurement	Species							
	Radish		Tomato		Lettuce		Barley	
	Exposed	Check	Exposed	Check	Exposed	Check	Exposed	Check
Germination (%)	100	100	50	100	90	70	80	100
Root length (cm)	6.9±1.4	5.4±1.9	3.9±1.9	4.1±1.9	2.7±1.4	4.7±0.8	6.7±3.6	9.8±2.8
Shoot length (cm)	3.7±0.7	3.8±1.1	2.3±1.3	2.7±0.9	1.6±0.7	3.3±0.3	1.8±1.3	3.1±1.4
	n.s[1]		n.s.		*		n.s.	
Shoot growth reduction (% of check)	2.4		4.6		52.4		41.4	

[1]n.s., not significant; *significant difference between check and exposed plants at 5% level

TABLE 21
EMERGENCE AND SEEDLING LENGTH OF FOUR SPECIES OF SEEDS EXPOSED TO 10 mg HF m^{-3} AND INCUBATED IN SOIL

Measurement	Species							
	Radish		Tomato		Lettuce		Barley	
	Exposed	Check	Exposed	Check	Exposed	Check	Exposed	Check
Germination (%)	90	90	45	80	65	70	50	90
Shoot length (cm)	46±10	50±8	50±11[1]	95±27[1]	43±8	40±18	204±22	169±30
	n.s[2]		**		n.s.		n.s.	
Growth reduction (% of check)	6.5		17.0		-9.6		-20.8	

[1]Tomato measurement made at four weeks, other species measured at two weeks
[2]n.s., not significant; **significant at 1% level by analysis of variance

TABLE 22
GERMINATION AND SEEDLING LENGTHS OF SIX SPECIES OF SEEDS EXPOSED TO 5.3 mg HF m^{-3} AND INCUBATED IN PETRI DISHES

Species	Control		Exposed to HF			
			Wetted		Soaked	
	Germination[1]	Length[2]	Germination	Length	Germination	Length
Barley	100	4.6 ± 0.6	10	1.4 ± 0.0	0	-
Bean	50	-	0	-	30	-
Lettuce	100	1.6 ± 0.3	0	-	0	-
Radish	100	3.6 ± 1.8	20	-	10	-
Squash	100	0.6 ± 0.4	60	0.4 ± 0.2	100	0.3 ± 0.2
Tomato	100	2.2 ± 0.7	0	-	0	-
Zinnia	80	2.0 ± 1.4	0	-	0	-

[1]Germination rate (%) at seven days after exposure
[2]Shoot lengths in cm of germinated seeds; mean and standard deviation of up to 10 seedlings; - indicates no shoots present

TABLE 23
EMERGENCE AND SEEDLING LENGTHS OF SIX SPECIES OF SEEDS EXPOSED TO 5.3 mg HF m^{-3} AND INCUBATED IN SOIL

Species	Control		Exposed to HF			
			Wetted		Soaked	
	Emergence[1]	Length[2]	Emergence	Length	Emergence	Length
Barley	80	18.7±2.5	90	19.0±2.2	30	19.7±1.0
Bean	70	13.9±2.3	70	14.2±2.4	60	13.1±2.6
Lettuce	90	3.8±0.4	50	4.9±1.4	20	3.0±1.2
Radish	90	4.4±0.4	90	4.3±0.6	70	5.1±1.0
Squash	80	17.1±2.0	100	20.2±2.4	70	17.1±2.5
Tomato	70	6.0±0.9	40	5.7±1.3	20	4.9±0.8
Zinnia	80	7.2±0.7	70	8.3±2.4	60	5.9±1.3

[1]Emergence (%) at seven days after exposure
[2]Total length in cm of seedlings emerging, measured at 14 days after treatment; mean and standard deviation of up to 30 seedlings

PHYTOTOXICITY OF JET FUEL

HYDROCARBON LITERATURE REVIEW

Literature on the effects of hydrocarbon oils on plants was reviewed and is presented in Appendix B. Entomologists in the 1940's and 1950's investigated the phytotoxicity of insecticidal oils. Kerosene, diesel fuel, and other hydrocarbons have been used as herbicides; their mode of action and plant susceptibility have been studied by weed scientists (Crafts and Reiber, 1948). Oil pollution researchers also investigated the toxicity of oil to plants, finding that the type and amount of oil involved, mode of application (vapor, spray, or dip), degree of weathering, environmental conditions, and the species and age of impacted plants all influenced phytotoxicity (Baker, 1970). Toxicity varied according to the concentration of low-boiling and unsaturated compounds, aromatics, and acids (Dallyn, 1953).

JET FUELS

Acquisition and Storage

Jet fuel supplied by the Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, included five gallons each of JP4-P and JP4-S. Fuel was stored in a locked, outdoor storage shed in the one-gallon drums in which they were delivered. One gallon gasoline cans of each material were kept in the laboratory in a fume hood for current experiments. Fuel was measured with graduated cylinders or with glass pipettes controlled by pipette bulbs. Soil and plants treated with fuel were kept outside for at least 24 hours to allow most noxious fumes to volatilize and dissipate.

Fuel Sprays

Fuel droplet size was determined by observing the patterns remaining on white paper situated below the spinning-disk applicator and was adjusted by varying the number of batteries powering the drive motor. Actual deposition of fuel was calculated by weighing spray collected on aluminum sheets (Figure 17). Deposition and droplet size appeared temperature-dependent.

Fuel Vapors

Vapor treatments were usually designated by the volume (ml) of jet fuel applied to the paper towel. A Beckman model 400 hydrocarbon (HC) analyzer was used to measure relative HC levels. Calibrated CO gas registered 5200 on the analyzer for 691 ppm CO. Three ml of fuel applied onto paper suspended in the 0.7-m^3 chamber produced a reading of 19,000 within four minutes of sealing the chamber. This level remained constant for at least 110 minutes. When the door was opened and the paper removed, the HC reading dropped to 500 within seven minutes and was 180 after 16 minutes.

After reaching equilibrium at 47,000 with 5 ml fuel, the chamber was exhausted for 15 minutes and resealed without the fuel-soaked paper. HC

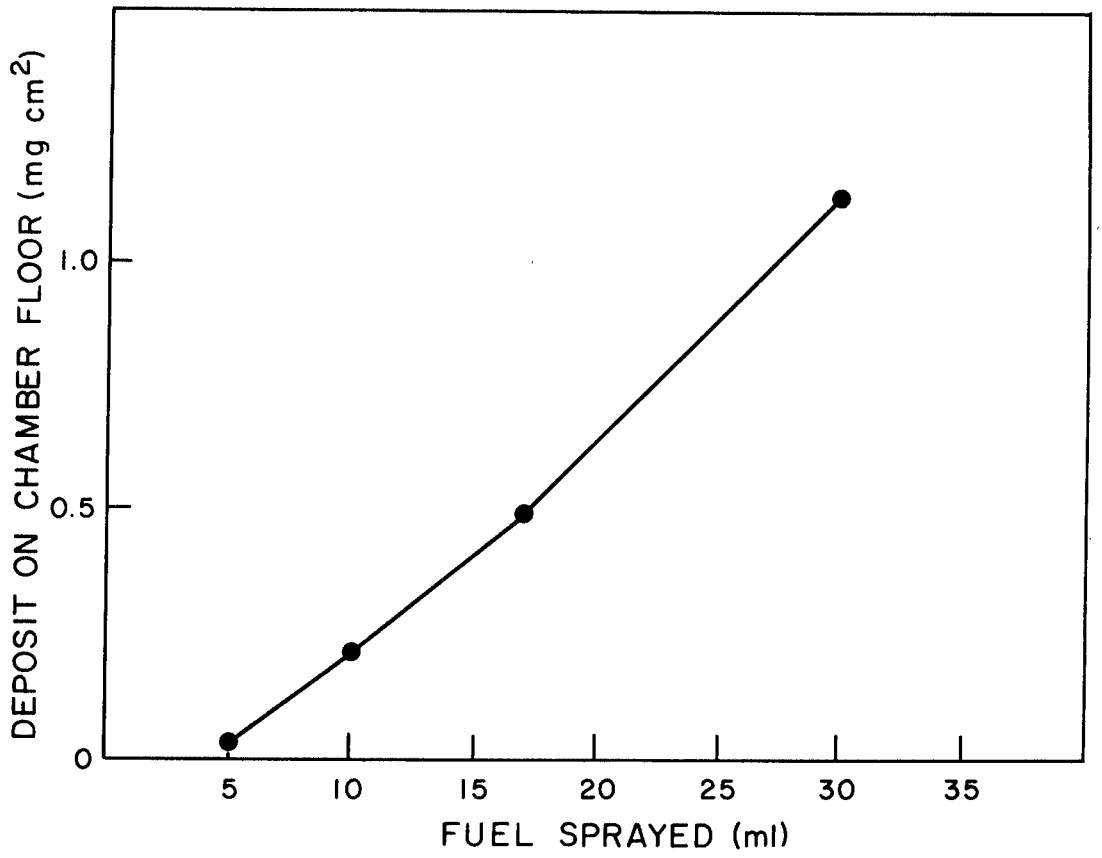


Figure 17. Deposition of JP4-P jet fuel spray using a spinning-disk applicator.

readings climbed from 730 to 2,330 in 10 minutes and continued to climb. Adsorbed hydrocarbons may be out-gassing from the walls of the chamber.

Hydrocarbon readings were compared for 5 ml of hexane or JP4-P fuel deposited on the paper (Figure 18). The HC reading stabilized within six minutes. A wet spot remaining on the paper still smelled of fuel after 15 minutes. Readings increased with the volume of hexane applied. HC readings with hexane were greater than those of the calibration gas, and were not within the linear range for that gas (Table 24).

The HC analyzer was useful for comparing relative amounts of fuel-derived hydrocarbons, but was not adequately calibrated for measuring jet fuels.

Fuel Drenches

Fuel used as drench treatments was recorded as volume (ml) applied to soil. The fuel was either applied directly to the soil surface or was mixed into the soil matrix by transferring a cup or pot of soil (ca. 500 g) to a clean one-gallon can, adding the jet fuel and rolling the sealed can to mix fuel and soil thoroughly. Mixing allowed more accurate characterization of fuel per unit of soil (ml cm^{-3} or ml g^{-1}).

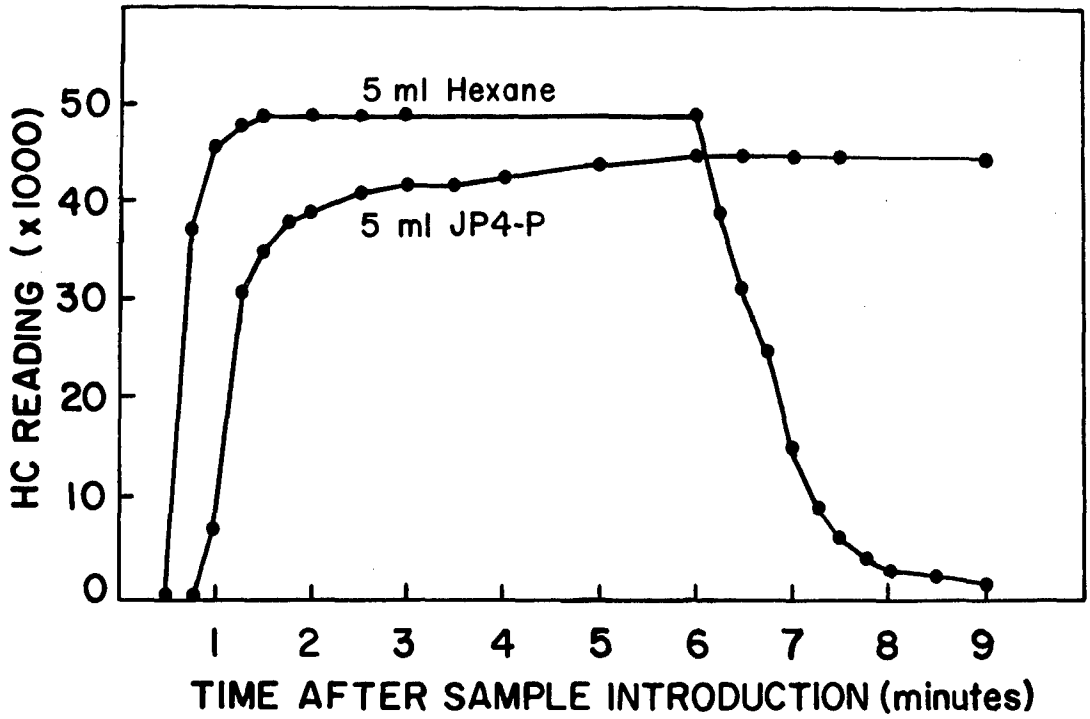


Figure 18. Relative hexane and JP4-P fuel concentrations measured with an hydrocarbon analyzer. Chamber door was opened at six minutes during hexane trial.

TABLE 24
HEXANE MEASUREMENTS WITH THE HYDROCARBON ANALYZER

Hexane applied to absorbant paper (ml)	HC monitor readings (x1000)	Hexane concn (ppm)
Calibration gas	11.0	691
1	26.0	1633
2	33.0	2073
4	42.0	2638
8	48.5	3015

PLANT RESPONSES TO FUELS

Response to Sprays

Groups of plants were exposed to JP4-P jet fuel at one of four spray concentrations (total volume sprayed) in the 0.66-m^3 Lucite chamber. Plants were returned to glasshouse benches and observed for injury on days following exposure. Three days after exposure, visible injury was evaluated on the 1-12 grading scale (Table 25). Ten days after exposure, plant fresh weights were measured and means of treated plant heights and weights were compared with those of controls (Table 26).

Leaves seemed differentially sensitive to fuel sprays with youngest leaves being most tolerant. The fresh weight of plants exposed to the

TABLE 25
VISIBLE INJURY ON PLANTS THREE DAYS AFTER EXPOSURE TO JET FUEL SPRAYS

Species	Age when treated (days)	Number leaves per plant	Spray treatment (ml) 0	5	10	17	30
Squash[1]	25	3	1.0	1.5	1.6	5.4	9.3
Squash[2]	18	2	1.0	1.2	1.0	1.5	7.6
Pea[1]	25	8	1.0	1.0	1.1	11.6	11.9
Barley[2]	18	3	1.2	1.2	1.1	2.3	3.5
Corn[2]	18	3	1.0	1.0	1.1	2.3	3.6
Lettuce[1]	32	4	1.0	1.1	1.0	1.3	1.6
Radish[1,3]	25	6	1.0	1.2	1.5	4.8	8.5
Bean[1]	18	2	1.0	2.4	8.9	11.8	12.0
Tomato[1]	32	5	1.1	1.5	2.0	7.6	10.0
Carrot[1]	32	3	1.0	1.0	1.0	1.0	1.0

[1]Mean of four plants
[2]Mean of 12 plants
[3]Injury recorded five days after treatment

TABLE 26
PERCENT FRESH WEIGHT OF PLANTS 10 DAYS AFTER EXPOSURE TO JET FUEL SPRAYS

Species	Age when treated (days)	Spray treatment (ml) 5	10	17	30
Squash[1]	25	102[3]	98	97	56
Squash[2]	18	103	88	81	47
Pea[1]	25	102	88	43	36
Barley[2]	18	112	106	92	91
Corn[2]	18	94	109	68	77
Lettuce[1]	32	116	83	85	66
Radish[1]	25	111	112	79	64
Bean[1]	18	109	64	45	49
Tomato[1]	32	104	102	95	39
Carrot[1]	32	89	101	120	102

[1]Mean of four plants
[2]Mean of 12 plants
[3]Fresh weight as percent of weights of unexposed plants

smallest dose appeared greater than the controls. Differences in sensitivity to the sprays among species were noted (Table 27). Bean and pea plants were most susceptible to the fuel, whereas carrot was most tolerant, an expected reaction of umbelliferous plants to oils.

The fuel spray experiment was repeated but only leaves at least 85% expanded were graded since partially (less than 15%) expanded leaves appeared less susceptible to fuel injury. Comparison of injury on similar leaves was summarized (Table 28). Average injury for the two experiments was plotted for the nine species tested (Figure 19). Plants were weighed 10 days after treatment. Comparisons between species were possible by calculating weights as percentages of controls. The differences in weights due to spray dose were significant for either test and for the combination of the two (Table 29). Species differences were also noted (Figure 20) and were the same as presented above: carrot and barley were tolerant and tomato, pea, and bean plants were sensitive to fuel sprays. In both tests and with all species (except carrot) spraying more fuel resulted in greater growth reduction (Table 29).

Response to Vapors

A glass rod installed across the inside of the Lucite chamber supported absorbent paper on which 5-20 ml of JP4-P jet fuel was applied after plants were sealed inside the chamber. Stirring paddles mixed air and vapor.

The plants were removed from the sealed chamber after two hours and graded for injury two to five days later (Table 30). Each fuel level (except 20 ml) was repeated on different days and the injury ratings were

TABLE 27
RELATIVE SENSITIVITY RANKINGS FOR PLANTS EXPOSED TO JET FUEL SPRAYS

Visible injury on fully expanded leaves		Growth inhibition compared to control
	Resistant	
Carrot		Carrot
Barley		Lettuce
Radish		Barley
Squash (25 day)		Corn
Lettuce		Squash (18 day)
Corn		Squash (25 day)
Tomato		Radish
Squash (18 day)		Tomato
Pea		Pea
Bean		Bean
	Sensitive	

TABLE 28
PLANT INJURY FROM JET FUEL SPRAYS IN TWO TRIALS

Species	Leaves[1] evaluated (no.)	Spray treatment during trials 1 and 2									
		0 ml		5 ml		10 ml		17 ml		30 ml	
		1	2	1	2	1	2	1	2	1	2
Barley	2	1.3[2]	1.1	1.3	1.2	1.2	1.1	2.9	1.3	4.1	4.7
Carrot	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lettuce	3	1.0	2.8	1.2	2.8	1.0	7.4	1.6	9.1	4.5	10.2
Corn	2	1.0	1.1	1.0	1.1	1.2	2.7	3.0	4.8	4.6	8.4
Squash	2	1.0	1.2	1.5	1.2	1.7	2.9	4.2	5.2	8.2	9.0
Radish	2	1.5	1.1	1.4	1.1	1.4	4.0	4.9	7.2	10.0	10.5
Tomato	1	1.0	1.0	1.8	1.3	3.0	5.3	8.0	10.4	12.0	12.0
Pea	5	1.0	1.5	1.4	2.5	3.0	10.7	12.0	12.0	12.0	12.0
Bean	2[3]	1.0	1.0	2.4	8.0	8.9	10.4	11.8	11.5	12.0	12.0
Average injury		1.20		1.84		3.77		6.22		8.23	

[1]Leaves used for evaluation did not include cotyledons or leaves less than 85% expanded at time of spraying
[2]Injury average of four plants on 1-12 scale where 1 is no injury
[3]Primary leaves

TABLE 29
RELATIVE PLANT WEIGHTS (% of controls) ONE WEEK AFTER JET FUEL SPRAYS IN TWO TRIALS

Sprayed (ml)	Trial 1 (%)	Trial 2 (%)	Trials 1 + 2 (%)
0	100 WX	100 W	100 W
5	104 W	91 WX	98 W
10	92 X	82 X	87 X
17	81 Y	71 Y	76 Y
30	61 Z	49 Z	56 Z

[1]Means in columns followed by the same letter are not statistically different at 1% level by Duncan's new multiple range test

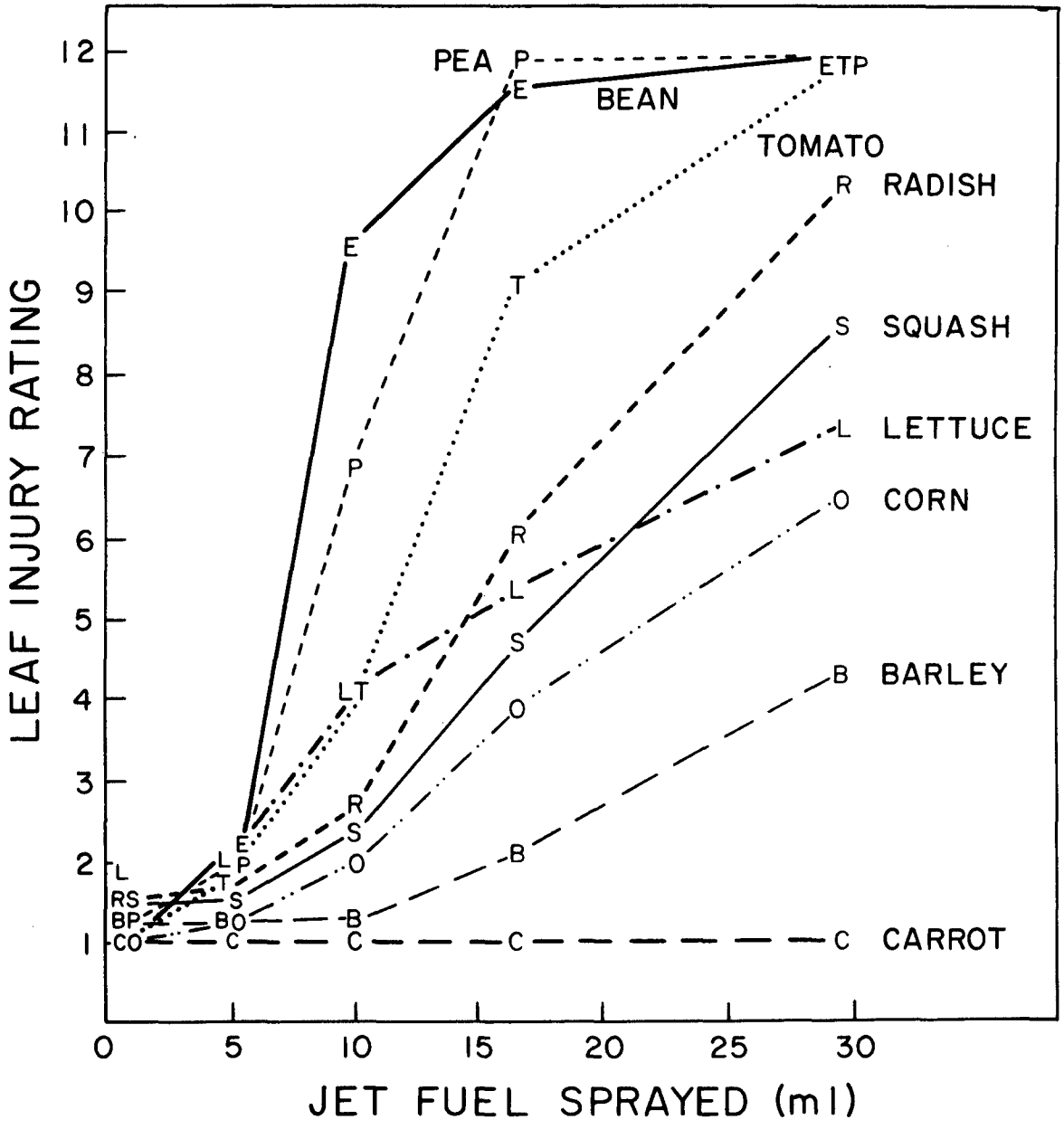


Figure 19. Leaf injury on nine plant species sprayed with JP4-P jet fuel.

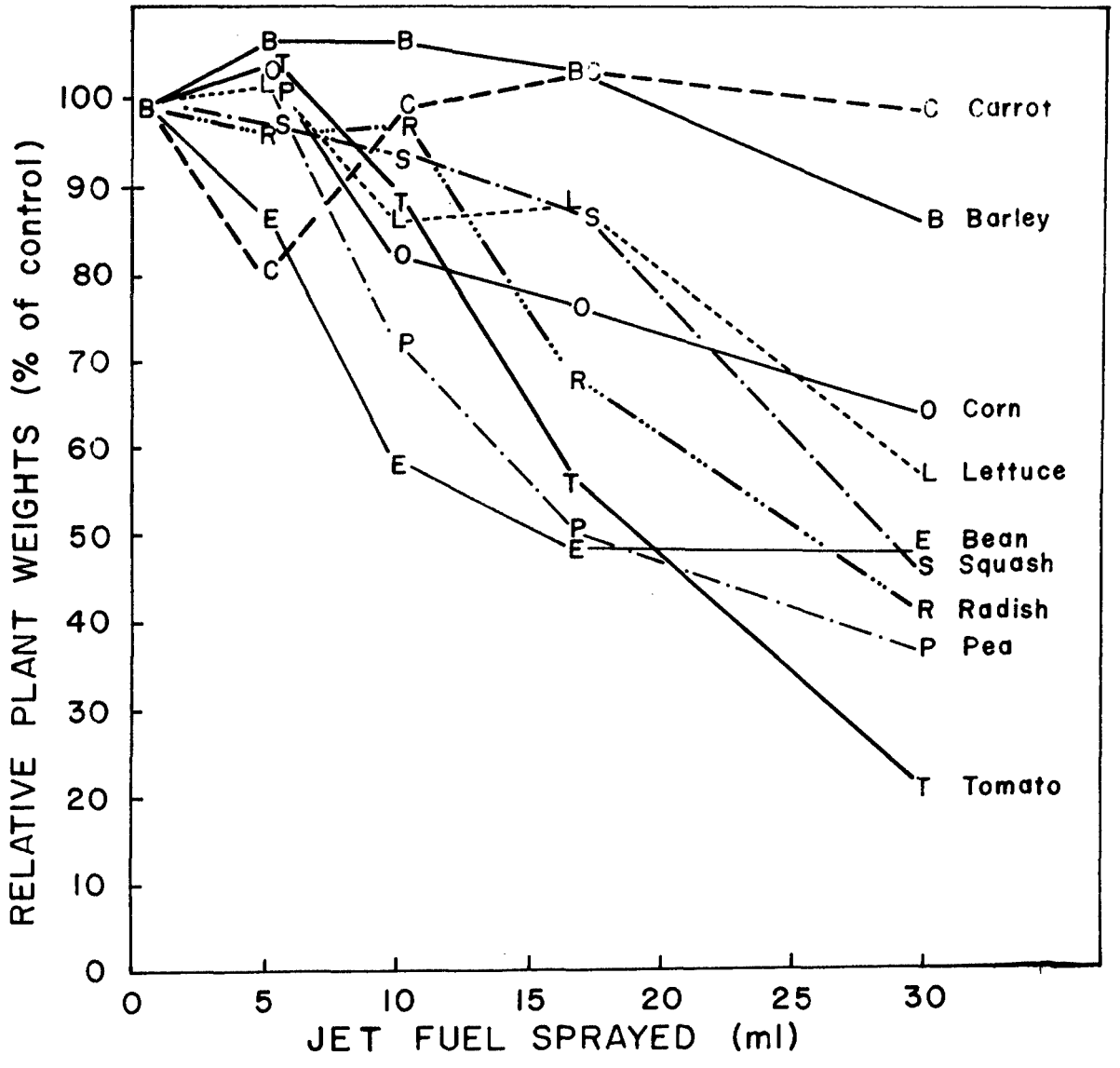


Figure 20. Relative weights of nine plant species assessed 10 days after spraying with JP4-P jet fuels.

TABLE 30
INJURY ON PLANTS EXPOSED TO JET FUEL VAPORS FOR TWO HOURS

Species	Leaves[1]	Fuel applied to absorbent paper (ml) 5	7.5	10	15	20
Barley	2	1.0[2]	1.2	1.6	2.9	5.1
Carrot	1	1.0	1.0	1.0	1.0	1.0
Lettuce	3	1.0	1.0	1.3	4.2	2.8
Corn (Young)	2	1.0	1.1	1.0	2.2	8.3
Corn (Old)	3	1.2	1.1	1.5	2.6	10.6
Squash	1	1.0	1.0	1.0	4.8	8.0
Radish	2	4.2	5.4	10.2	7.6	12.0
Tomato	1	1.0	3.8	4.0	7.5	10.5
Pea	3	1.0	5.2	10.0	11.8	12.0
Bean (Young)	1	1.4	4.2	4.5	3.6	12.0
Bean (Old)	1	1.2	6.0	9.8	9.5	11.8
Average injury		1.36	2.82	4.17	5.25	8.55

[1]Number fully expanded leafsets at time of exposure
[2]Injury graded using 1-12 Horsfall-Barratt scale where 1 is no injury

averaged. Injury varied between the two replicates but always increased with amount of fuel applied to the paper. Species sensitivity to vapor corresponded to the sensitivity of the same species to injury from fuel sprays.

Comparison of Shale and Petroleum Fuels as Sprays and Vapors

A multifaceted experiment was designed to compare jet fuels derived from shale with those derived from petroleum. Corn and bean plants represented monocot and dicot species which were relatively sensitive to fuel. Seedlings were either one or two weeks of age at exposure. Fuel was applied to the test plants as aerosol sprays or vapors from deposits on absorbent paper. For either exposure system, two fuel concentrations were used and the two species received different doses, depending on their tolerance. The low fuel level was 5 ml for beans and 10 ml for corn, while the high level was 10 ml and 20 ml for the two species, respectively. Four bean and eight corn plants were used in each treatment. Plants were in the exposure chamber for less than five minutes for the spray and for two hours for the vapor treatments. Injury on each treated plant was estimated by two independent observers (Table 31).

A multiple-factor analysis of variance revealed certain relationships among the factors (Table 32). No significant differences in plant injury

TABLE 31
INJURY ON BEAN AND CORN LEAVES EXPOSED TO VAPOR OR SPRAY APPLICATIONS OF JET FUEL DERIVED FROM SHALE OR PETROLEUM AND GRADED BY TWO OBSERVERS

Species	Fuel level[1]	Fuel source[2]	Vapor[3]				Spray			
			Young[4]		Old		Young		Old	
			1[5]	2	1	2	1	2	1	2
Bean	Low	Shale	1.4[6]	1.4	2.5	2.7	2.2	2.8	2.2	2.2
		Petrol	1.1	1.1	1.2	1.0	1.0	1.0	1.0	1.0
	High	Shale	5.0	4.6	4.8	4.9	7.2	7.6	8.8	8.5
		Petrol	3.8	3.8	3.2	3.4	6.2	6.0	8.0	7.6
Corn	Low	Shale	2.3	1.8	1.6	1.3	2.6	2.6	3.0	4.7
		Petrol	2.0	1.6	1.7	1.6	1.6	2.0	2.4	2.7
	High	Shale	4.7	4.5	4.9	5.1	5.5	4.9	5.0	4.7
		Petrol	4.5	4.3	5.8	5.8	5.0	4.2	4.7	4.2

[1]Fuel level was different for the two species: Low = 10 ml and 20 ml and High = 5 ml and 10 ml for bean and corn, respectively
[2]JP4 fuel was derived from shale or petroleum
[3]Fuel applied to suspended paper created vapors; sprays produced with spinning-disk applicator
[4]Young plants were 13 days old and old plants were 20 days old
[5]Two observers graded the plants independently
[6]Injury, rated on 1 to 12 scale; means of eight young bean, 12 old bean, or 24 corn leaves

were observed between the grading of the two observers (O) or the age of the plants (A). Certain factors, however, were significant. The spray treatment was more injurious than fuel vapors; shale-derived fuel caused more injury than the petroleum-based fuel (D); and small fuel concentrations were less phytotoxic than larger concentrations (C). In two interactions that were significant, FxS and CxF, fuel form and fuel concentration were the important factors determining injury.

This experiment supported the hypothesis that shale fuel was more phytotoxic than petroleum-derived fuel. It also confirmed our beliefs that qualified graders will grade plants similarly. Although these spray treatments were more phytotoxic than the vapor treatments, direct comparisons were difficult since more fuel reached the plant during spraying (as both spray and vapor).

TABLE 32
ANALYSIS OF VARIANCE OF JET FUEL PHYTOTOXICITY TEST WITH MULTIPLE FACTORS

Source of Variation		df	Sum of squares	F-value
Observers (O)	(1, 2)	1	0.03	0.03
Age of plants (A)	(young, old)	1	2.21	2.54
Fuel form (F)	(vapor, spray)	1	17.74	20.36 ***[1]
Fuel derivation (D)	(shale, petroleum)	1	8.63	9.90 **
Fuel concentration (C)	(small, large)	1	188.72	216.51 ***
Species (S)	(corn, bean)	1	0.54	0.62
Observers x Species (OxS)		1	0.03	0.03
Observers x Form (OxF)		1	0.06	0.06
Age x Concentration (AxC)		1	0.17	0.20
Age x Form (AxF)		1	0.34	0.40
Age x Derivation (AxD)		1	0.00	0.00
Form x Derivation (FxD)		1	1.08	1.23
Form x Species (FxS)		1	6.96	7.98 **
Derivation x Species (DxS)		1	2.76	3.17
Concentrtion x Form (CxF)		1	4.15	4.76 *
Concentration x Derivation (CxD)		1	0.15	0.17
Error		47	40.97	
Total		63	274.54	

[1]Significance at 5%, 1%, and 0.1% levels for *, **, and ***, respectively

PHYTOTOXICITY OF JET FUEL DRENCHES

Seed Bioassays in Petri Dishes

Seeds make useful bioassay material; they can be selected for rapid growth and sensitivity to specific toxicants (Chang and Thompson, 1966). In an initial test, tomato seeds were incubated with jet fuel and water in plastic Petri dishes. Nine-cm Whatman No. 1 filter paper disks were fitted into both halves of the dishes. Two ml DDW were applied to the disks in one-half of the Petri plate and the disks in the other half received 0, 0.25, 0.50, or 1.00 ml JP4-P jet fuel plus enough DDW (2.00, 1.75, 1.50, or 1.00 ml) to equal 2 ml liquid. Only one of the Petri dish halves received fuel. Tomato seeds were placed in the bottom half in groups of 25 and the dishes were sealed with Parafilm strips. During incubation, water was added to the dish by piercing the Parafilm with a syringe and injecting DDW.

Seedlings were removed and measured after incubating in the dark for 13 days (Table 33). In the presence of fuel, seedling development was inhibit-

TABLE 33
GROWTH (cm) OF TOMATO SEEDLINGS INCUBATED IN PETRI DISHES WITH JET FUEL

Treatment					
Plate half	Fuel (ml)	Shoots (cm)	Roots (cm)	Total length (cm)	Shoot/ total ratio
Top	0.00	4.1 Y[1]	4.1 W	8.2 W	0.50
Top	0.25	0.9 Z	1.7 XY	2.6 XY	0.35
Top	0.50	0.9 Z	2.1 X	3.0 X	0.30
Top	1.00	1.1 Z	1.3 Y	2.3 Y	0.48
Bottom	0.00	4.5 Y	4.8 V	9.3 V	0.48
Bottom	0.25	1.0 Z	2.1 X	3.1 X	0.32
Bottom	0.50	0.9 Z	0.6 Z	1.5 Z	0.60
Bottom	1.00	1.0 Z	2.2 X	3.2 X	0.31

[1]Means in any column followed by the same letter(s) are not significantly different at 5% level by Duncan's new multiple range test

ed. Differences in initial amounts of water could have affected growth, but this would not adequately explain the decreases, since sufficient additional water was added during incubation. In general, shoots were inhibited slightly more than roots by the fuel treatments.

The relationship between fuel dose and seedling length reduction was not always linear. When fuel was in the top half of the Petri dish, fuel vapors reached the seeds; liquid fuel plus fumes reached seeds when the bottom filter disks were treated. No difference in seedling lengths was detectable when top and bottom treatments were compared.

Fuel appeared to kill seedlings. Seeds germinated and began to grow, then tissue became discolored, further growth ceased, and seedlings eventually decayed. An additional source of phytotoxicity may have been reaction products formed when plastic dishes were partially dissolved by fuel.

Seeds Grown on Contaminated Soil

Since soil is often contaminated with jettisoned fuel, seed bioassays of fuel were conducted using sterile soil mix. Polystyrene drinking cups (350 ml) were filled with soil which was allowed to absorb water through holes punched in the cup bottoms. JP4-P jet fuel was pipetted onto the moist soil surface. After 15 minutes, 25 tomato seeds were sown in each cup and covered with a thin layer of vermiculite. Shoots, roots, and number of leaves were measured 28 days after sowing (Table 34). The fuel reduced emergence slightly and caused a small reduction in seedling lengths. Soil seemed to moderate the effect of the fuel, although the warm glasshouse temperatures and comparatively large soil volume may have driven off or diluted the phytotoxic compounds.

TABLE 34
GROWTH (cm) OF TOMATO SEEDLINGS ON SOIL CONTAMINATED WITH JET FUEL

Fuel (ml)	Shoots (cm)	Roots (cm)	Total (cm)	Shoot/ total ratio	Germi- nation (%)	Leaves (no.)
0	3.7 B[1]	9.6 AB	13.3 B	0.28	100	3.2 C
1	3.3 B	11.2 B	14.5 B	0.23	80	2.5 B
2	2.7 A	9.1 AB	11.8 AB	0.23	76	1.8 A
5	2.4 A	7.0 A	9.4 A	0.26	84	1.8 A

[1]Means separated as in Table 33

Seedling Development after Rinsing Seeds with Fuels

Some reports indicate shale-derived fuel, JP4-S, may be more toxic to biological systems than the petroleum product, JP4-P (Kline and Jenkins, 1981). In this experiment, seeds were exposed briefly to liquid fuel of the two types and seed development was monitored.

Groups of 25 tomato and 10 squash seeds were shaken for 10 seconds in a glass vial containing 10 ml of JP4-P, JP4-S, or DDW. The seeds were washed onto a screen with a stream of DDW and then placed on filter paper in Petri dishes or on the surface of vermiculite in 350-ml cups. Water was added to the Petri dishes (tomato, 2 ml; squash, 3 ml) which then were sealed with Parafilm and stored in the dark at 24°C. The cups were kept in the glasshouse and watered as needed.

Emergence or germination and total seedling lengths were measured nine days after treatments for seedlings in Petri dishes and after 14 days for seedlings in vermiculite. The fuel had no effect on tomato germination (Table 35). Squash germination was reduced by the fuel treatment, particularly in those seedlings in vermiculite. Seedlings that developed after the brief fuel treatments were shorter compared to water controls (Table 36). The average reduction for Petri dish-incubated seedlings was 42% for tomato and 78% for squash. Seedlings were longer in vermiculite, partly because growing periods were extended five days. The reductions averaged 21% for tomato and only 8% for squash. No toxicity differences could be assigned to the two fuels from these tests, although seeds treated with shale fuel treatments yielded seedlings that were slightly shorter than petroleum fuel-treated seeds.

Seed Development in Contaminated Field Soil

The influence of soil type and fuel application technique in fuel drench experiments was considered. UC Mix II, a sterile, sandy soil blend,

TABLE 35
DEVELOPMENT OF SEEDS EXPOSED TO LIQUID JET FUELS

Species	Petri dish incubation[1]			Vermiculite incubation[2]		
	DDW	JP4-P	JP4-S	DDW	JP4-P	JP4-S
Tomato	100	100	100	88	90	88
Squash	70	20	10	95	80	80

[1]%-emergence nine days after treatment
[2]%-emergence 14 days after treatment

TABLE 36
TOTAL LENGTHS (in cm) OF SEEDLINGS EXPOSED
TO LIQUID JET FUELS

Species	Petri dish incubation[1]			Vermiculite incubation[2]		
	DDW	JP4-P	JP4-S	DDW	JP4-P	JP4-S
Tomato	78.2	48.8	41.6	83.5	172.5	59.6
Squash	10.1	2.6	1.8	267.2	249.0	245.6

[1]Length of seedlings harvested nine days after treatment
[2]Length of seedlings harvested 14 days after treatment

has been described (Table 2). A sandy clay loam was collected from a University of California, Riverside, field plot and coarsely sifted through 0.125-inch mesh screen. For mix treatments, 25 ml water and 5 ml JP4-P jet fuel were added to gallon cans containing 500-600 g soil and the cans were rolled. The thoroughly mixed soil was transferred back to the pots. For drip treatments, fuel was applied to the surface of soil in 4-in pots. Control pots were untreated. Fifteen tomato seeds were planted in each pot. The pots remained in a tray of water overnight, then were surface-watered as needed.

Emergence was observed daily. Forty-four days after sowing, seedlings were counted (Table 37) and shoot lengths were measured (Table 38). Lengths were reduced in the presence of fuel. When compared to UC mix, field soil inhibited seedling emergence and shoot lengths even without fuel treatment. This may be attributed to such factors as better soil structure and

TABLE 37
EMERGENCE OF TOMATO SEEDLINGS GROWING IN SOIL TREATED WITH 5 ml JET FUEL

Soil treatment	Soil source: Field soil	Soil source: UC Mix
No fuel	12.5 ± 0.6[1] (83%)[2] A[3]	13.8 ± 1.0 (92%) A
Fuel dripped	7.8 ± 1.7 (52%) B	9.5 ± 2.6 (63%) B
Fuel mixed	9.5 ± 1.3 (63%) B	10.2 ± 1.0 (68%) B

[1]Number seeds emerging per pot; mean and standard deviation of four pots of 15 seeds each
[2]Percent emergence
[3]Means in any column followed by the same letter are not significantly different at 5% level by Duncan's new multiple range test

TABLE 38
SHOOT LENGTHS (in mm) OF TOMATO SEEDLINGS GROWING IN SOIL TREATED WITH 5 ml JET FUEL

Soil Treatment	Soil source: Field soil	Soil source: UC mix
No fuel	435 ± 113[1] A[2]	1125 ± 426 A
Fuel dripped	457 ± 89 A	1092 ± 348 A
Fuel mixed	261 ± 69 B	554 ± 109 B

[1]Shoot height in mm of emerged seedlings; mean and standard deviation of up to 55 seedlings
[2]See note 3, Table 37

more available nutrients in the prepared mixture. Fuel was more injurious to seedling emergence when mixed into soil than when dripped onto the surface. Growth of surviving seedlings was inhibited by the fuel regardless of how it was applied, suggesting that fuel at the surface (drip) may form a chemical barrier through which the emerging seedling must pass. The seed would germinate normally below the barrier. Germinating seeds contacted inhibitory materials earlier where fuel was incorporated into the soil than where it was on the surface.

Sensitivity of Seeds to Jet Fuel

Seeds of seven plants were tested for emergence in soil contaminated with fuels. Fifteen seeds each of alfalfa, bean, corn, radish, squash, sudan grass, and tomato were sown per four-inch pot of soil mix. Three pots of each species were untreated controls and three pots were treated with 3 ml of JP4-P jet fuel dripped on the soil surface. Numbers of emerging seeds in each pot were counted periodically (Table 39). Final counts were made 14 days after sowing and percent emergence was calculated as a proportion of seeds emerging in controls.

One object of this experiment was to identify rapid-growing indicator plants sensitive to fuel-contaminated soil. Emergence of all species tested was reduced by the presence of 3 ml of fuel in ca. 400 cm^3 soil. Sudan grass grew rapidly in untreated soil and appeared most sensitive since no plants emerged in treated soil. Alfalfa and corn developed rapidly but were less sensitive to the fuel than sudan grass. Although squash and tomato were fuel-sensitive, these species took longer to grow even in fuel-free soil. Radish developed rapidly and was the most tolerant species tested.

In a second experiment, five species were screened for their sensitivity to jet fuels. The species could be divided into monocot-dicot groups and by type of photosynthesis. Wheat and sorghum were C3 and C4 monocots, respectively; alfalfa, cotton, and sunflower were C3 dicots. Five 4-inch pots per species were filled with 550 g soil, sown with 10 seeds per pot, and covered with additional soil. Treatments consisted of dripping 3 ml of JP4-P jet fuel onto the soil surface. Shoot heights were measured 14 days after sowing seeds (Table 40).

In most cases, fewer seedlings emerged in treated soil (cotton was a very tolerant exception). Seedlings which did grow had reduced shoot lengths compared to seedlings from untreated soil, but only for alfalfa and sorghum were these differences statistically significant. Analysis of wheat seedlings was hampered because so few treated seeds developed. Cotton and sunflower seedling lengths were not affected by fuel treatment.

From these experiments the relative sensitivities of several species were estimated on the basis of percent-emergence compared to controls (Table 41). Many of the more sensitive species were monocots; the C3-type plants appeared more tolerant.

Recovery of Fuel-contaminated Soil

Two tests were conducted to observe whether fuel could be removed from or neutralized in contaminated soil.

In the first test, 5 ml of JP4-P jet fuel was pipetted onto soil in 350 ml perforated cups. The soil was then flushed with 0 to 400 ml tap water and 25 tomato seeds were sown on the surface. The larger quantities of flush water compacted the soil and this factor was not controlled in this experiment. Emerged seedlings were counted and measured 28 days after treatment (Table 42).

TABLE 39
EMERGENCE OF SEEDLINGS OF SEVEN SPECIES GROWING IN SOIL CONTAMINATED WITH JET FUEL

Species[1]	Days after sowing								
	3	4	5	6	7	8	10	11	14
Sudan Grass	0[2]	0	0	0	0	0	0	0	0
Tomato	0	0	0	0	0	0	0	0	0
Corn	0	0	0	0	0	0	0	5	5
Pinto Bean	0	0	0	0	0	0	0	0	17
Squash	0	0	0	0	0	0	15	20	29
Alfalfa	0	0	0	6	9	12	15	19	30
Radish	0	0	13	39	54	57	66	66	66

[1]Species arranged in order of increasing tolerance
[2]Emergence in treated pots sown with 45 seeds per species as percent of emergence in untreated pots

TABLE 40
GROWTH OF SEEDLINGS OF FIVE SPECIES IN SOIL CONTAMINATED WITH JET FUEL

Species	Soil treatment	Emergence[1]		Shoot length	
		Number	% of Control	mm	% of Control
Wheat	Fuel	2	4	179 ± 212[2]	58
	No fuel	47		310 ± 33	
Sorghum	Fuel	4	8	129 ± 95	38
	No fuel	48		339 ± 72	
Sunflower	Fuel	26	76	156 ± 33	91
	No fuel	31		172 ± 29	
Alfalfa	Fuel	37	84	139 ± 29	91
	No fuel	44		153 ± 25	
Cotton	Fuel	36	106	231 ± 21	104
	No fuel	34		222 ± 34	

[1]Emergence of seedlings per pot; mean of five pots of 10 seeds each
[2]Shoot length in mm of emerging seedlings; mean and standard deviation of up to 48 seeds

TABLE 41
RELATIVE SENSITIVITY OF SEEDS OF TEN SPECIES TO MODERATE CONCENTRATIONS OF JET FUEL IN SOIL

Sensitive (0-5% Emergence)	Intermediate (6-29% Emergence)	Tolerant (30-100% Emergence)
Sudan Grass	Bean	Alfalfa
Tomato	Squash	Radish
Wheat		Sunflower
Corn		Cotton
Sorghum		

TABLE 42
GROWTH OF TOMATO SEEDLINGS ON FUEL-CONTAMINATED SOIL FLUSHED WITH WATER

Water flush (ml)	Emergence (%)	Lengths			Leaves (no.)
		Tops (cm)	Roots (cm)	Total (cm)	
0	92[1]	2.2[2] BC[3]	7.3 B	9.5 C	1.8 AB
25	72	1.7 A	5.1 AB	6.8 AB	1.6 A
50	76	2.0 ABC	3.6 A	5.7 A	1.6 A
100	88	2.0 AB	5.7 AB	7.7 ABC	1.9 AB
200	80	2.7 D	6.1 B	8.8 BC	2.4 BC
400	52	2.5 CD	7.6 B	10.0 C	2.3 C

[1]Percent of 25 sown seeds which emerged
[2]Mean lengths of emerged seedlings
[3]Means in columns followed by the same letters are not significantly different at 5% level by Duncan's new multiple range test

Emergence was reduced with the largest (400 ml) water treatment; total seedling length was greatest with the 0 or 400 ml flushes and number of leaves increased with amount of water. The hypothesis that flushing soil with water reduced phytotoxicity of fuel (by removing toxic substances) was not completely acceptable, since emergence was reduced. Some soil "cleansing" was evident from increased plant lengths and number of leaves in the 400 ml treatment, but considerable interference in normal growth probably occurred from soil compaction, texture change, and nutrient leaching.

The second test involved waiting a certain period between treating soil and sowing seeds. Four-inch pots containing soil were wetted with tap water, allowed to drain, and 0, 5, or 10 ml of JP4-P jet fuel was applied to

the soil surface. Barley or tomato seeds were sown at a depth of 3 to 5 mm at zero, three, or seven days after fuel treatment.

Barley and tomato seedlings were counted and measured 10 and 31 days after seeds were sown, respectively (Tables 43 and 44). For both species the emergence and seedling heights decreased as fuel treatment increased. Only with barley seeds did the delay in planting increase percent emergence. Barley seedling height, tomato emergence, and tomato heights were not influenced by the delay in sowing.

Effect of Fuel on Seedling Development

A test was conducted to verify the greater toxicity of fuel mixed into the soil as compared to surface application and to examine the nature of the toxicity. JP4-P jet fuel was mixed by adding 3 ml to 550 g soil and rolling it in a one-gallon can. Soil was transferred to a plastic four-inch pot with a small reserve set aside to cover 10 sorghum or alfalfa seeds sown on the soil surface. For the drip method, seeds were sown in 550 g soil, covered with soil, then 3 ml fuel was pipetted on the surface starting at the center and spiraling outward. Controls had no fuel added. Each treatment was replicated five times. Pots were placed on trays of water and incubated in the glasshouse. Seedling emergence, root lengths, and shoot lengths were recorded 14 days after sowing.

Both species were inhibited when grown in soil contaminated with jet fuel (Table 45). Fuel mixed into soil reduced seedling emergence and plant lengths more than when dripped onto the surface. When shoot-root ratios were compared, the drip and control treatments were not significantly different for either species at the 5% level (Table 45). In each case, however, the ratio for the mix treatment was greater. Although both shoots and roots were shorter with the mix treatment, greatest reduction occurred with root lengths. Direct effects on roots were expected since the toxicant was applied via the soil. Based on emergence expressed as percent of controls, sorghum was more sensitive than alfalfa to either treatment (Table 46). Alfalfa was more sensitive when lengths were compared.

Dose-response of Sorghum

A dose-response experiment was conducted to determine fuel concentrations necessary to inhibit sorghum seedling growth. Seeds were sown in four-inch pots containing 500 g of soil. Zero to 16 ml JP4-P jet fuel per pot was dripped onto the soil surface or was mixed with soil prior to potting and sowing. Emergence and shoot lengths were measured 14 days after treatment.

Three groups were identified based on mean number of emerging seedlings per pot (Table 47). Seedling emergence for statistical group A did not differ significantly from the control at the 5% level. Intermediate and severe inhibition of emergence were shown by groups B and C, respectively. As could be predicted from earlier findings, fuel mixed into soil had greater inhibition on plants than the same amount dripped onto the soil surface. Four ml fuel mixed into the soil produced the same amount of phytotoxicity as dripping on 8 ml.

TABLE 43
GROWTH OF BARLEY SEEDLINGS FROM SOIL PREVIOUSLY TREATED WITH JET FUEL

Fuel (ml)	Delay between treating soil and sowing seeds (days) 0	3	7
Emergence[1] (%)			
0	90 ± 7	93 ± 8	83 ± 9
5	0	63 ± 12	83 ± 9
10	1 ± 3	42 ± 49	70 ± 50
Shoot heights[2] (cm)			
0	16.6 ± 3.7	12.9 ± 2.4	12.9 ± 1.0
5	0	12.8 ± 2.4	8.0 ± 2.2
10	9.0 ± 0	12.1 ± 1.2	9.4 ± 1.6

[1]Percent seedlings emerged of 80 sown; mean and standard deviation of four pots
[2]Shoot heights of emerged seedlings, mean and standard deviation of up to 80 plants (four pots of 20 seeds sown per pot)

TABLE 44
GROWTH OF TOMATO SEEDLINGS FROM SOIL PREVIOUSLY TREATED WITH JET FUEL

Fuel (ml)	Delay between treating soil and sowing seeds (days) 0	3	7
Emergence[1] (%)			
0	92 ± 9	98 ± 3	90 ± 10
5	82 ± 9	85 ± 4	84 ± 12
10	78 ± 6	70 ± 15	70 ± 4
Shoot heights[2] (cm)			
0	9.7 ± 2.7	10.8 ± 2.5	7.7 ± 2.1
5	6.4 ± 1.8	6.3 ± 1.7	5.4 ± 1.6
10	5.0 ± 0.9	4.8 ± 1.3	4.5 ± 1.8

[1,2]See Table 43

TABLE 45
GROWTH OF ALFALFA AND SORGHUM IN SOIL CONTAMINATED WITH 3 ml JET FUEL

Species / Measure		Soil treatment: No fuel	Drip	Mix
Alfalfa				
Emergence	(no.)[1]	8.8 ± 1.3	5.6 ± 1.1	3.0 ± 1.2
	(%)[2]	86	62	30
Lengths[3]: (cm)	Shoot	5.7 ± 0.08	5.1 ± 0.8	3.9 ± 0.4
	Root	7.5 ± 3.4	6.6 ± 2.8	3.6 ± 1.5
	Total	13.2 ± 3.2	11.8 ± 2.8	7.4 ± 1.7
Mean shoot/root ratio		0.764	0.772	1.072
Sorghum				
Emergence:	(no.)	8.4 ± 1.1	7.4 ± 1.8	3.6 ± 2.1
	(%)	84	74	36
Lengths: (cm)	Shoot	10.2 ± 2.1	8.2 ± 2.1	4.4 ± 2.5
	Root	21.9 ± 6.5	17.5 ± 6.5	5.7 ± 3.5
	Total	32.1 ± 6.7	25.7 ± 5.8	10.2 ± 3.8
Mean shoot/root ratio		0.467	0.471	0.774

[1]Number of seedlings emerged per pot of 10 seeds sown; mean and standard deviation of five pots
[2]Percent emerged of 50 seeds sown
[3]Lengths (in cm) of seedlings which emerged per pot; mean and standard deviation of five pots of seedlings

TABLE 46
RELATIVE EMERGENCE AND LENGTHS OF ALFALFA AND SORGHUM FROM SOIL CONTAMINATED WITH JET FUEL

Species		Emergence[1] (%)	Total length[2] (cm)
Alfalfa	Drip	72	89
	Mix	35	57
Sorghum	Drip	88	80
	Mix	43	32

[1,2]See Table 45

TABLE 47
EMERGENCE OF SORGHUM 14 DAYS AFTER SOIL TREATMENTS WITH JET FUEL

Fuel treatment (ml)	Application method	
	Mixed into soil	Dripped onto soil
0.0	12.0 ± 1.4[1] A[2]	13.2 ± 1.8 A
0.5	11.2 ± 0.8 A	13.0 ± 1.2 A
1.0	12.8 ± 1.9 A	12.8 ± 1.8 A
2.0	11.6 ± 2.3 A	13.2 ± 0.8 A
4.0	4.2 ± 1.8 B*[3]	12.8 ± 0.5 A
8.0	1.0 ± 1.2 C	4.2 ± 3.0 B*
16.0	0.0 C	0.0 C

[1]Number of seedlings emerging per pot; mean and standard deviation of five pots of 15 seeds each
[2]Means in same column followed by the same letter were not significantly different at 5% level by Duncan's new multiple range test
[3]Threshold values are followed by *

Duncan's new multiple range test was used to separate the mean seedling lengths for the treatments at the 5% level (Table 48). Five groups were separated in the mixing treatment, but only two in the drip treatments. Four ml produced a measurable effect on plant length whereas 1 ml inhibited emergence. In drip treatments, 8 ml fuel was the smallest amount which inhibited emergence or seedling lengths.

Fuel mixed into the soil was phytotoxic at lower levels than when the same amount was dripped onto the soil surface. Less fuel was needed to inhibit length reduction than to reduce seed emergence, particularly when fuel was mixed into the soil. Drip treatments probably did not affect seedling length as much as the mix because the fuel remained closer to the soil surface and did not influence deeper root development.

Horizontal Movement of Fuel Toxicity in Soil

The horizontal movement of jet fuel, or of its phytotoxic component, was detected by noting the effect on seed germination. A wooden tool was used as a template to form a grid of 9 x 12 holes in the soil contained in plastic 17 x 17 x 2-inch flats. The tool had 108 pegs each 6-mm diameter, 25-mm deep, and 15-mm apart (Figure 21). Two grids were formed in each flat of soil and three sorghum seeds were sown in each hole. JP4-P jet fuel was applied by pipette to a portion of the seeded area and seeds were covered with soil. The application pattern was either along the line formed by a row of seeds or in a rectangular area enclosing 12 or 16 holes (Figure 22). Either 10 or 20 ml of fuel was applied. For the rectangular areas,

TABLE 48
TOTAL LENGTHS (in mm) OF SORGHUM SEEDLINGS FROM SOIL
TREATED WITH JP4-P JET FUEL

Fuel treatment (ml)	Application method			
	Mixed into soil		Dripped onto soil	
	Emerged[1] (no.)	Lengths[2] (mm)	Emerged (no.)	Lengths (mm)
0	65	322 ± 102 A[3]	65	329 ± 77 A
0.5	58	338 ± 105 A	65	329 ± 73 A
1.0	66	280 ± 89 B*[4]	65	338 ± 76 A
2.0	63	227 ± 82 C	66	351 ± 67 A
4.0	41	119 ± 48 D	52	358 ± 70 A
8.0	25	69 ± 29 E	64	203 ± 90 B*
16.0	1	13 ± 0 E	2	107 ± 34 B

[1]Number of seedlings emerging from 75 sown
[2]Total length of emerging seedling; mean and standard deviation of number emerged
[3,4]See Table 47, notes 2 and 3

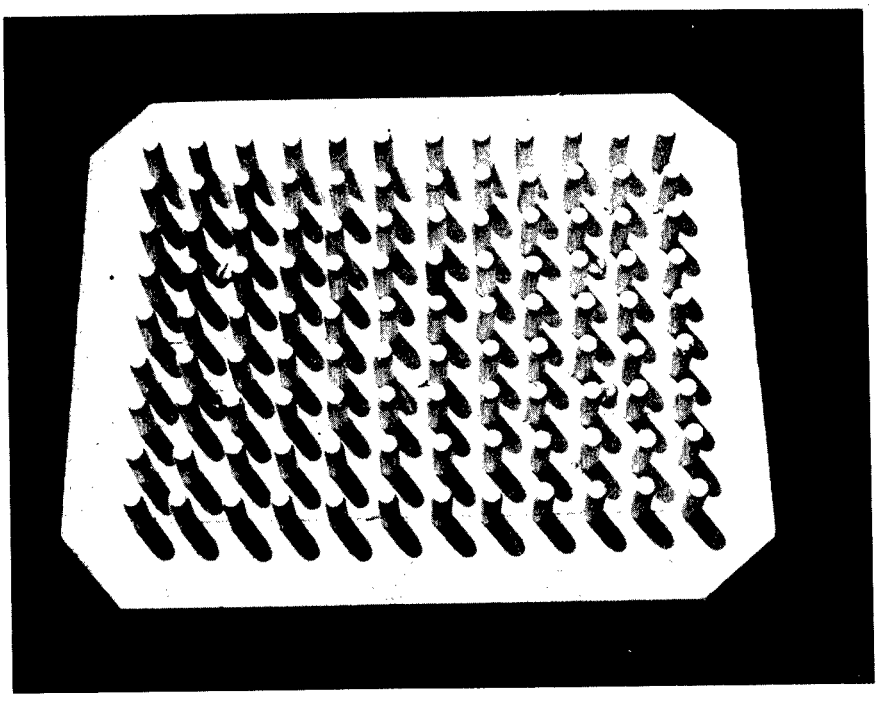


Figure 21. Wooden tool used to form uniform grid of holes for planting seeds in soil.

application was expressed as 1.5 ml cm^{-2} or 1 ml cm^{-2}. Flats were set on trays of water and incubated in the glasshouse.

Phytotoxic effects were expressed by the lack of seedling emergence (Figure 22). Movement of fuel usually extended one row to either side of the treated row, at least to 1.5 cm but less than 3 cm from the point of application. Seeds in the treated area never emerged. Seeds in 42% and 0% of the holes emerged in rows adjacent to the 10-ml and 20-ml treatments, respectively. When fuel was concentrated into a smaller area, no seeds within the area emerged. Fuel movement was not greater than 1.5 cm when the treated area was in the center of the grid. Seeds 3 to 4.5 cm away from the application site did not emerge when the treated area was in a corner of the grid, perhaps because movement was influenced by the sides of the flat.

Some horizontal movement of jet fuel or of its phytotoxic component occurred in these tests since emergence of seeds was prevented adjacent to sites of applications.

Vertical Movement of Fuel in Soil

Vertical movement of the phytotoxic component of jet fuel was investigated using eight columns, each constructed of 2-foot long (61-cm) by 3-inch (7.6-cm) diameter PVC tubing screened on the bottom (Figure 23). The columns were firmly packed with soil. A 7-cm filter paper disk was placed on the upper soil surface and 45 ml of JP4-P fuel was poured onto the column. Fuel was absorbed into the soil within 30 seconds. Columns were placed upright in a shaded part of the glasshouse. After seven days, the soil was removed from the columns by forcing it out with a closely-fitting plunger (Figure 24) and cutting off 5-cm-deep sections. Each soil section was placed in a four-inch pot and sown with 15 sorghum seeds. Emerging plants were counted and measured 14 days after sowing (Table 49). Only in soil from 5 to 15 cm from the column top was seed emergence reduced. As with other fuel movement work, seedling length was a more sensitive measure of phytotoxicity than emergence. In this case, the material significantly reduced lengths of seedlings growing in soil from the column surface to a depth of 25 cm with greatest reduction occurring near the surface (Table 50).

Enough phytotoxic material remained in portions of a column of soil seven days after treatment to inhibit emergence and reduce growth of seedlings. Emergence of seedlings in the first 5-cm section, although reduced, may not be significantly inhibited because of greater contact with air and subsequent volatilization.

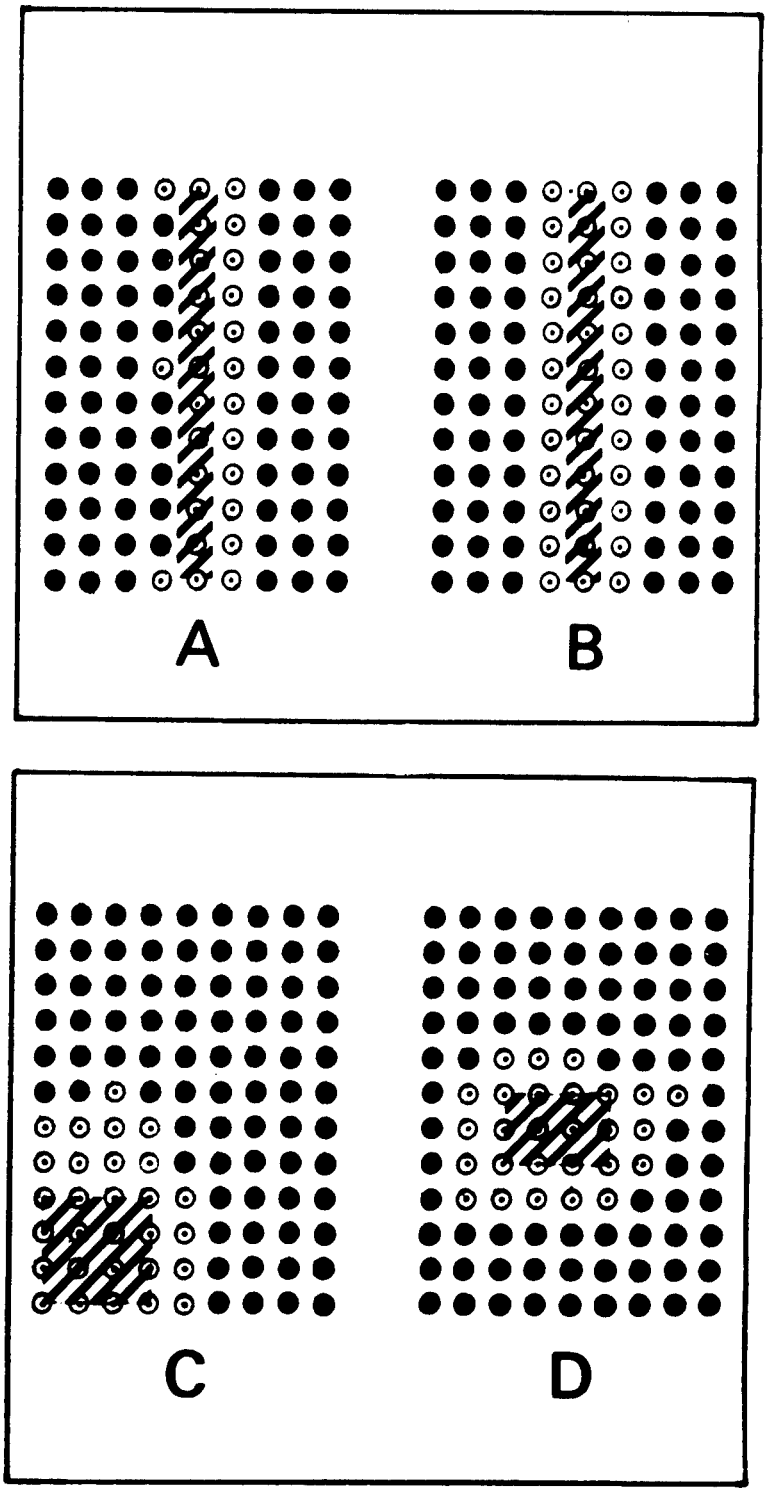


Figure 22. Patterns depicting areas of fuel application (cross-hatching) and seedling emergence (●) or non-emergence (⊘). Upper flat: Linear application at 10 (A) and 20 ml (B). Lower flat: Area application at 1 ml cm^{-2} (C) and 1.5 ml cm^{-2} (D).

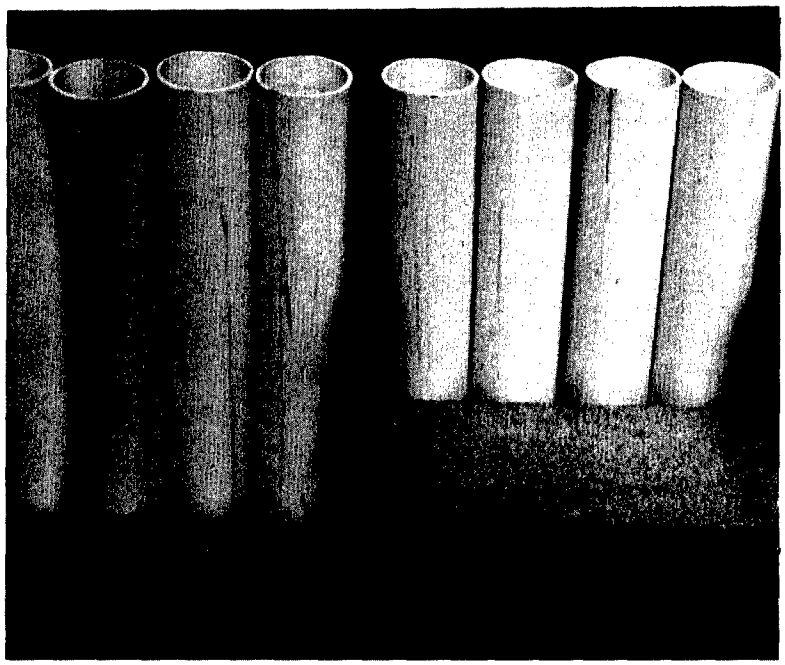


Figure 23. PVC columns used to detect movement of fuel in soil.

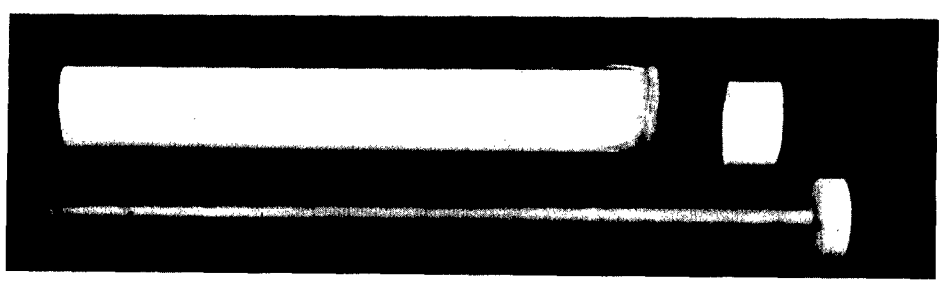


Figure 24 (left). PVC column, 5-cm wide PVC form, and steel plunger. Plunger forces soil from column; form measures 5-cm wide soil sections.

TABLE 49
GROWTH OF SORGHUM IN SOIL SECTIONS FROM COLUMNS TO WHICH 45 ml JET FUEL WAS APPLIED

Depth of section from top[1] (cm)	Emergence[2] (no.)		Total lengths[3] (cm)	
	Fuel	No Fuel	Fuel	No Fuel
5	7.2 ± 3.3	11.0 ± 1.8	149 ± 31	* 332 ± 58
10	7.8 ± 1.0 *[4]	12.2 ± 1.0	140 ± 29	* 317 ± 77
15	6.8 ± 1.7 *	11.8 ± 1.5	154 ± 27	* 337 ± 78
20	9.2 ± 2.2	11.5 ± 0.6	183 ± 50	* 318 ± 86
25	11.2 ± 0.5	11.8 ± 1.7	218 ± 68	* 299 ± 54
30	13.8 ± 0.5	12.2 ± 2.2	302 ± 58	298 ± 76
35	12.0 ± 1.6	11.0 ± 2.4	320 ± 65	309 ± 75
40	13.5 ± 1.3	11.0 ± 1.4	330 ± 61	330 ± 65
45	12.5 ± 1.0	12.5 ± 1.0	338 ± 61	324 ± 77
50	12.5 ± 1.7	12.5 ± 2.1	326 ± 63	325 ± 73

[1]Reference is from column surface to lower face of the section (i.e., 5 is the 0-5 cm sample)
[2]Number of seedlings which emerged per pot of 15 sown; mean and standard deviation of four pots
[3]Total mean length and standard deviation of seedlings emerging
[4]*denotes significant difference between Fuel and No fuel data at 5% level

TABLE 50
REDUCTIONS OF GROWTH OF SORGHUM IN SOIL SECTIONS FROM COLUMNS OF SOIL TREATED WITH JET FUEL

Depth of section[1] (cm)	Reductions (%)	
	Emergence	Total lengths
5	34.6	55.1
10	36.1	55.8
15	42.4	54.3
20	20.0	42.5
25	5.1	27.1

[1]See note 1, Table 49

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APPENDIX A

PICTORIAL KEYS OF FOLIAR INJURY

BACKGROUND AND RATIONALE

During the course of these studies, it was useful to devise pictorial keys with which injured leaves could be compared. Such assessment keys were not new (James, 1971); plant disease keys have been useful to plant pathologists for years. Our keys were based on the pre-transformed injury rating scale of Horsfall (Horsfall and Barratt, 1945; Horsfall and Cowling, 1978). The rating scale is pre-transformed to compensate for the tendency of the eye to attach greater importance to small differences in injury at lowest and highest levels.

CONSTRUCTION OF KEYS

Leaf outlines of the six species used in the HF dose-response test, namely tomato, barley, bean, radish, lettuce, and zinnia, were traced on acetate sheets and portions of the outlines were darkened to represent typical necrosis using injured leaves as guides. Photographs of actual leaf injury were recorded (Figures A1, A2). A Li-Cor Model L13000 portable leaf area meter (Lambda Instrument Corp., 4421 Superior St., Lincoln, Nebraska) accurately measured the darkened areas on the acetate models. Minor adjustments were made to the drawings so figures were available for injury levels 2-11 for all six species (Figures A3, A4, and A5).

USE OF KEYS IN GRADING

In use, leaves from exposed plants were compared to the keys and assigned a score from 1 to 12. Although most injury was foliar necrosis, the grader could indicate chlorosis or glazing on the grading sheet. The keys were usable for foliar injury caused by either fuel or gaseous HF, and may be applied to any assessment of leaf area.

Two graders independently using this grading system achieved the same injury ratings.

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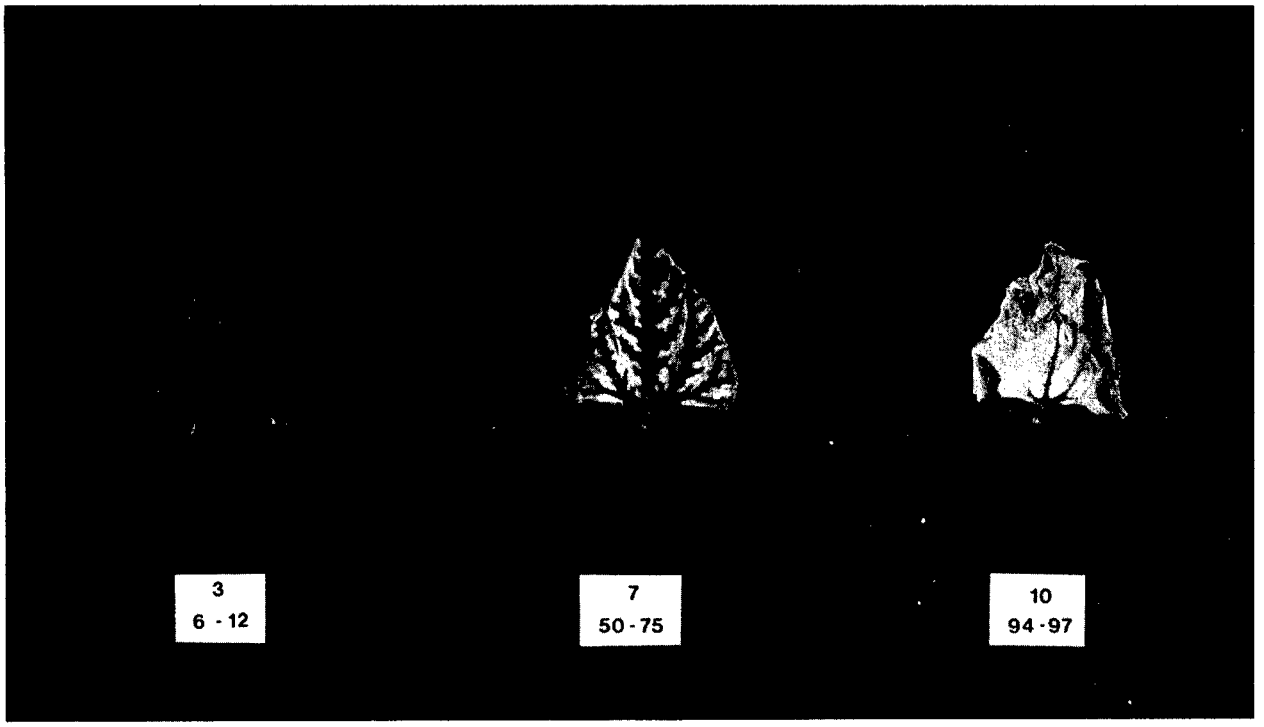

Figure A1. Rating grade and percent necrosis range for three pinto bean leaves injured by exposure to HF gas.

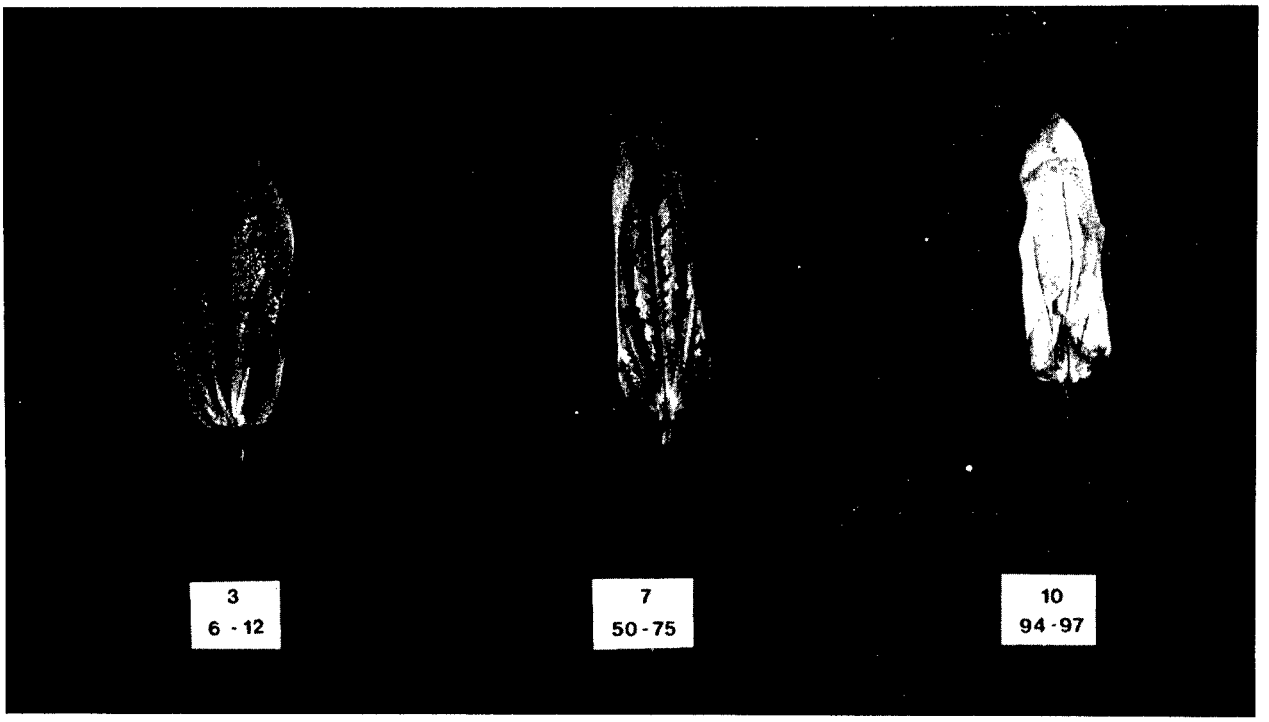

Figure A2. Rating grade and percent necrosis range for three zinnia leaves injured by exposure to HF gas.

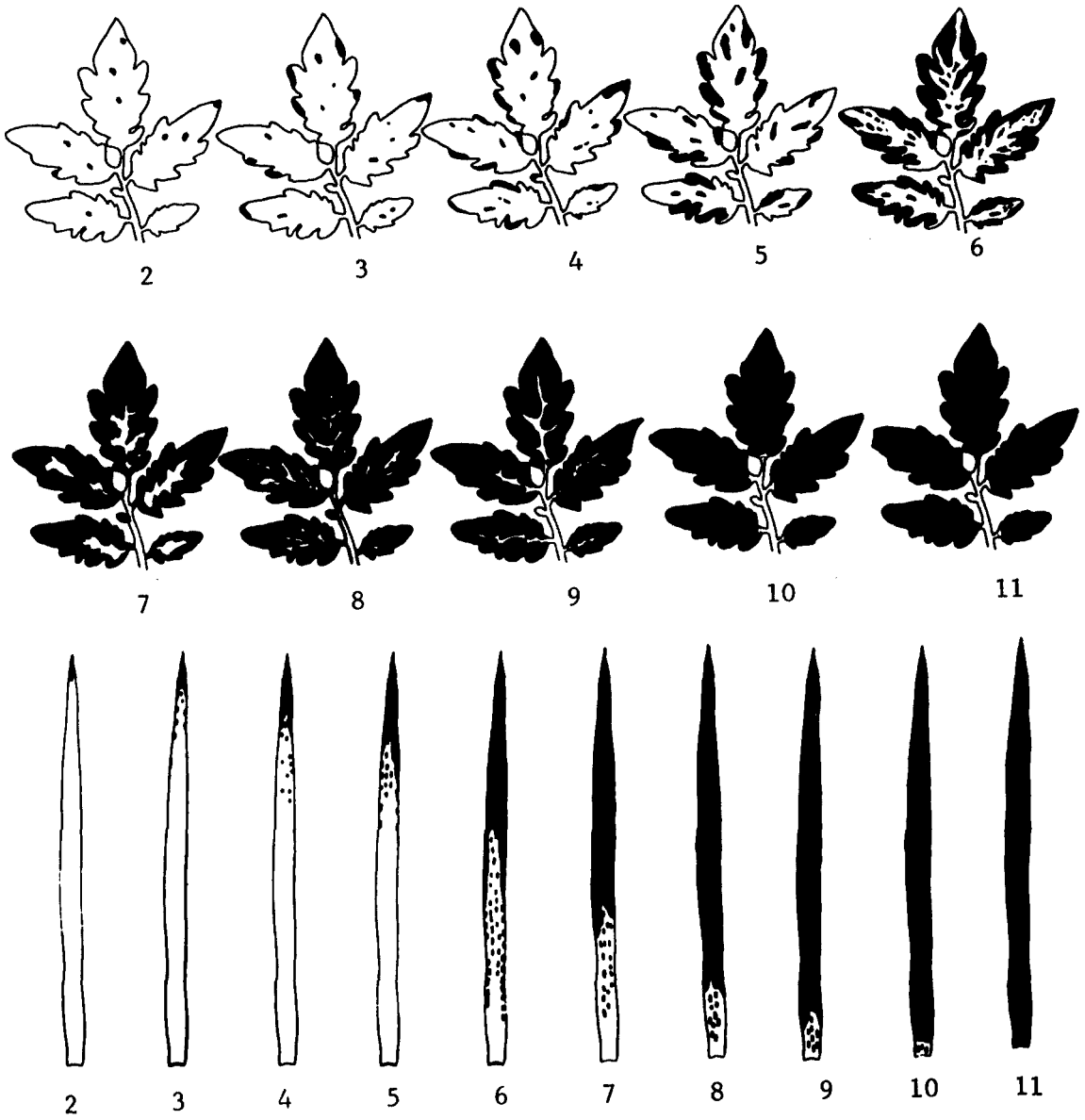

Figure A3. Pictorial key for necrotic injury on tomato (top two rows) and barley leaves. Percent of leaf necrosis (darkened area) is average for grading scores of 2 = < 3%, 3 = 3-6%, 4 = 6-12%, 5 = 12-25%, 6 = 25-50%, 7 = 50-75%, 8 = 75-87%, 9 = 87-94%, 10 = 94-97%, and 11 = > 97%. Not illustrated are 1 = no injury (0%) and 12 = death of leaf (100%).

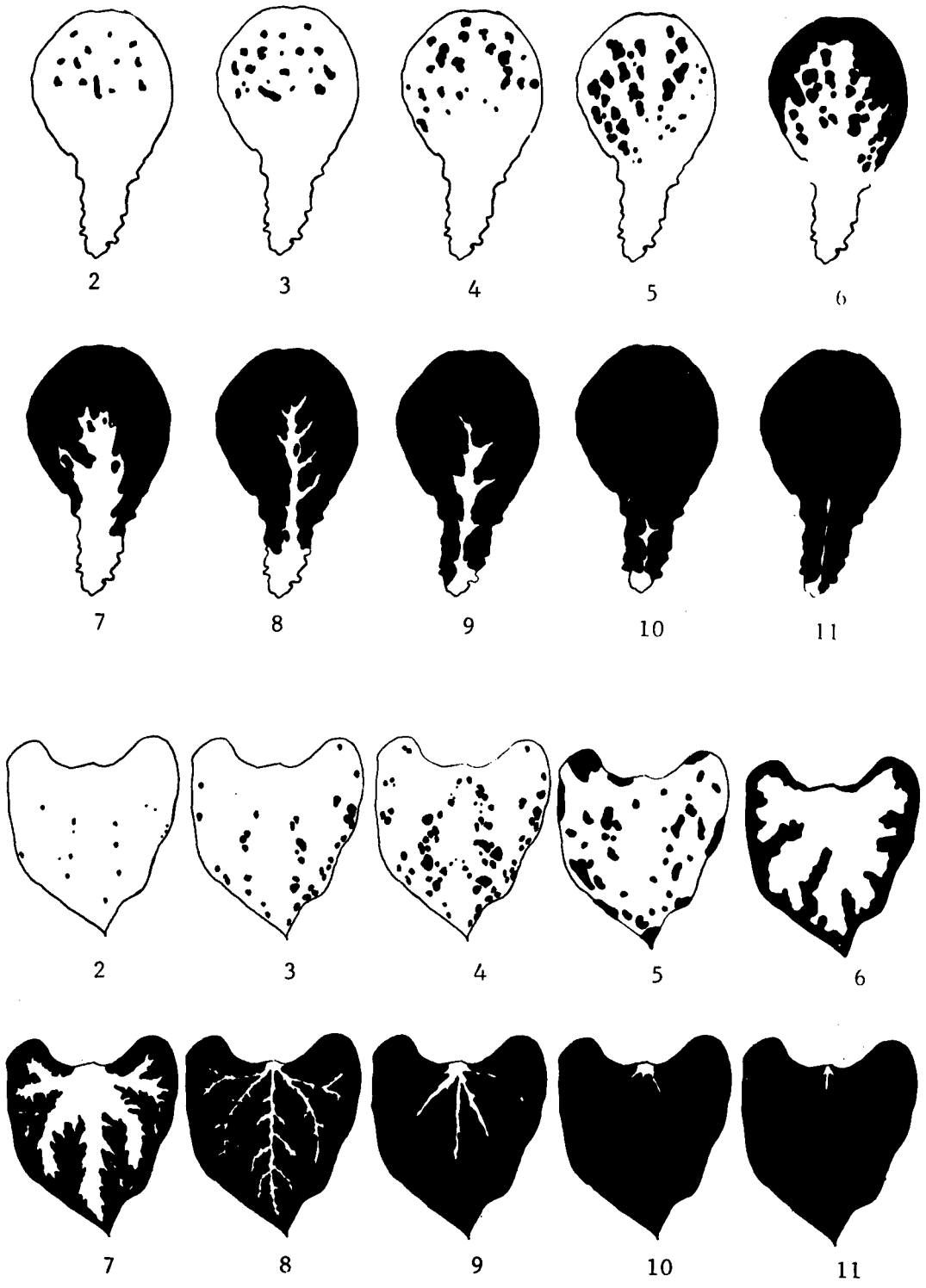

Figure A4. Pictorial key for necrotic injury on lettuce (top two rows) and pinto bean leaves. See Figure A3 for code.

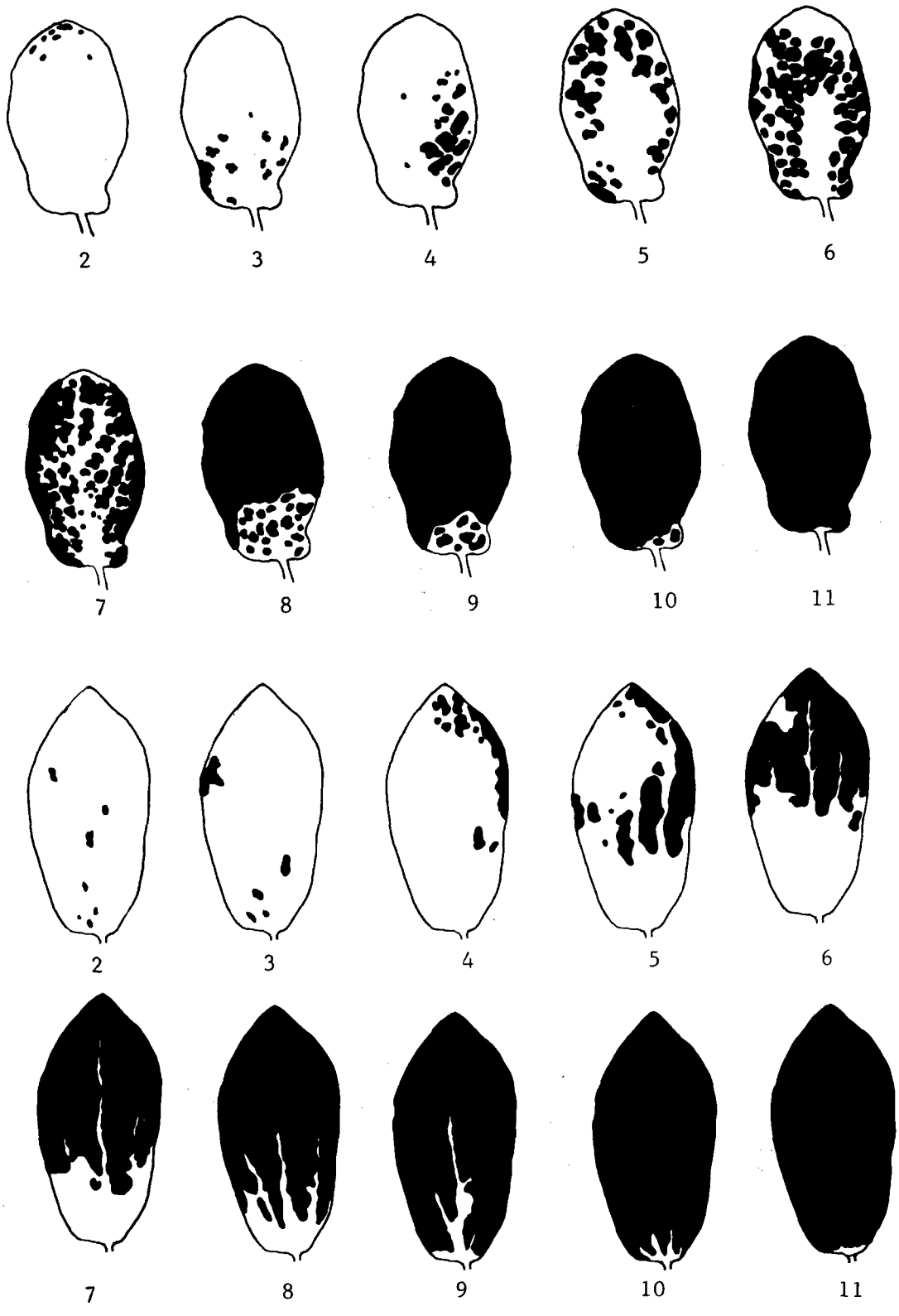

Figure A5. Pictorial key for necrotic injury on radish (top two rows) and zinnia leaves. See Figure A3 for code.

APPENDIX B

LITERATURE REVIEW OF HYDROCARBONS AS PHYTOTOXICANTS

FUELS IN THE ENVIRONMENT

Jettisoning unburned jet fuel is practiced when aircraft weight must be reduced for operational or emergency reasons. The extent of Air Force fuel jettisoning has been reviewed by Clewell (1980). When dumping is carried out at sufficient altitude, most of the released fuel evaporates and only a minor fraction reaches the ground in liquid form. Fuels may also enter the environment through spills during terrestrial operations. Little has been published about the effects of jet fuel on crops and other vegetation, but other petroleum products have been used for decades for agricultural purposes. Diesel oil, kerosene, and similar petroleum materials have been used to kill all vegetation along roadsides, ditches, and fences. Stove oil and Stoddard solvent have been used as selective herbicides, mainly in umbelliferous crops. Other oils have been used as insecticides (De Ong et al., 1927), fungicides (Calpouzos, 1966, 1969), and as pesticide carriers or adjuvants. In the latter applications plant injury must be avoided and research has focused on finding effective oils with low phytotoxicity. Further information has come from investigations on the effects of oil spills and oil pollution of water on plants. A comprehensive literature review has been published (Baker, 1970).

FORMS OF OIL PHYTOTOXICITY

Acute Injury

Two main types of oil injury to plants have been distinguished. Acute injury is caused by low-boiling hydrocarbons and consists of a rapidly developing necrosis. Dark green spots, due to cell sap leakage into the intercellular spaces, appear within minutes or hours after treatment, followed by loss of turgor, chlorophyll destruction, and death of the tissue within a few days.

Chronic Injury

Chronic injury, caused by heavy oils, is characterized by slowly developing chlorosis and retardation of growth. Leaf drop and reductions in the soluble-solid content of the fruit have been noted in citrus (Riehl et al., 1954). Low-volume oil applications to banana leaves result in small, elongated, rust-colored streaks appearing within two to three weeks (Calpouzos, 1969).

PHYTOTOXICITY OF OIL FRACTIONS

Entry of Certain Fractions

Oils tend to spread over plant surfaces to form thin films which readily penetrate stomata in contrast to droplet-forming aqueous solutions and oil-in-water emulsions which do not penetrate. Penetration of oil through the cuticle may occur to a limited extent, but plant parts with well-developed cuticles and few stomata, such as the upper surface of apricot leaves (Van Overbeek and Blondeau, 1954) or the succulent leaves of sedum are not easily penetrated or injured. Injury was much reduced on plants sprayed in the dark when their stomata were closed (Van Overbeek and Blondeau, 1954). On the other hand, Dallyn (1953) concluded that acutely toxic oil entered leaves indiscriminately at the point of contact, while nontoxic oils penetrated largely or entirely through the stomata. Gudin et al. (1976) found that photosynthetic inhibition following application of two oils did not depend on whether the stomata were open or closed, suggesting that these oils penetrated through the interstomatal regions of the cuticle. Rate of penetration may depend on the viscosity of the oil. Oils of low toxicity spread throughout the plant, from shoot to root and vice versa, presumably through intercellular spaces (Minshall and Helson, 1949a; Dallyn, 1953). Oil injury was not systemic, however, and Dallyn (1953) concluded that acutely toxic oil moved only short distances away from the site of application.

Toxicity of Certain Fractions

Gray and De Ong (1926) suggested that a good indicator of oil phytotoxicity is the sulfonatable fraction (i.e., the unsaturated compounds). Oils < 10% sulfonatable were safe (Calpouzos, 1969). This gives only a rough indication, however, since the sulfonation test does not distinguish between aromatic and olefinic compounds, and the aromatic fraction is most responsible for toxicity (Havis, 1950; Dallyn, 1953). In fungicidal oils, however, the saturated fraction was the toxic fraction causing flecking on banana leaves (Calpouzos, 1969). Upon exposure to light or after artificial oxidation, many oils increase in toxicity. This is caused by the formation of acids and, to a lesser extent, peroxides (Crafts and Reiber, 1948; Johnson and Hoskins, 1952; Van Overbeek and Blondeau, 1954). The olefinic fraction is especially susceptible to this process (Havis, 1950). Such an increase in toxicity may also take place after oils have been applied to the plants (Dallyn, 1953).

Toxicity of Liquids and Vapors

Plants and plant parts have been exposed to pure hydrocarbons in vapor (Currier, 1951; Ivens, 1952; Currier and Peoples, 1954) or liquid form (Crafts and Reiber, 1948; Havis, 1950; Van Overbeek and Blondeau, 1954; Boyles, 1976) to study the toxicity of individual components of oils. Toxicity of liquid hydrocarbons increased in the order: (1) paraffins, (2) olefins and naphthenes, and (3) aromatics (Havis, 1950; Van Overbeek and Blondeau, 1954; Boyles, 1976). Phytotoxicity of vapors of paraffins, olefins, and aromatics was roughly the same (Ivens, 1954).

Relationship of Toxicity to Hydrocarbon Molecular Chemistry

Dipping plant parts in liquid hydrocarbons showed that within each class of compounds the smallest molecules (lowest boiling point and lowest viscosity) have the highest toxicity (Van Overbeek and Blondeau, 1954; Boyles, 1976). In spray tests, however, very volatile compounds were not the most toxic since they evaporated too rapidly. Thus, toxicity first increased with increasing boiling point, then decreased (Leonard and Harris, 1950; Havis, 1950). In the vapor phase, too, the toxicity on a molar basis increased with boiling point up to a "cut-off point" at about 170°C and rapidly decreased above this value (Ivens, 1952).

ACTION OF HYDROCARBON FUELS

Biochemical Basis for Phytotoxicity

In a discussion of the mode of action of oils, a distinction may be made between acute and chronic injury. Low boiling compounds cause very rapid cell collapse and leakage of cell constituents. The action is so rapid that a biochemical mode of action was thought unlikely (Currier, 1951). Ivens (1952) demonstrated that the degree of vapor saturation (thermodynamic activity) required for toxicity was between 0.1 and 1.0, suggesting also that the toxicity depends on physical properties of the toxicant molecules rather than on their participation in a biochemical reaction. Van Overbeek and Blondeau (1954) suggested that the hydrocarbon molecules solubilize into the lipid bilayer of the plasma-membrane and disrupt it. Bulky or bent molecules would be more toxic than straight-chain molecules because they cause more disruption of the lipid bilayer. For compounds with such a physical mode of action, the thermodynamic activity in the external phase is a useful measure of the toxicity since, at equilibrium, it is equal to the activity in the biophase (Ferguson's principle, Albert, 1979, pages 543-556). Boyles (1976) suggested that the saturation concentration in a biophase diminishes logarithmically with chain length and that the hydrocarbon concentration in the membrane is directly proportional to the rate of membrane breakdown. Therefore, as chain length increased in an homologous series, toxicity increased because of a more favorable biophase/external phase partition coefficient. At a certain chain length, saturation of the biophase is required to produce injury and higher boiling compounds were not toxic (Ivens, 1952; Boyles, 1976). The partitioning of a compound into the biophase depends on the nature of the compound, of the biophase, and of the external phase. When a toxicant was diluted with water (emulsified) or nontoxic hydrocarbons, the external phase was modified and the toxicity altered, a phenomenon noted by many authors (Dallyn, 1953; Havis, 1950; Currier and Peoples, 1954; Boyles, 1976).

Physiological Responses of Plants to Fuels

Physiological responses following oil applications include reduced transpiration, reduced photosynthesis and either reduced or increased respiration. For a detailed discussion and references see Baker (1970). Both low boiling hydrocarbons and heavier oils cause these responses, but

with the former the plant tissue either is subsequently killed or--in resistant plants or with less toxic oils--the processes return to normal within hours or days (Minshall and Helson, 1949b). Effects of heavier oils persist for long periods; recovery of photosynthesis (Riedhart, 1964) and transpiration (Riehl and Wedding, 1959; Riehl et al., 1958) was directly correlated with dissipation of the oil deposit. Chronic plant injury has been attributed to interference with leaf gas exchange and the subsequent effects on transpiration and photosynthesis. Such effects could be due to the oil film acting as a physical barrier or to a physiological effect on the behavior of the stomata (Dallyn, 1953), but this mechanism has remained controversial (Baker, 1970; Gudin et al., 1976). Van Overbeek and Blondeau (1954) believed that chronic injury, at least in its first stages, was caused by the same membrane disruption process (albeit slower) that caused acute injury. Interference with water balance of the plant (Minshall and Helson, 1949a) or the reduction in respiration after treatment with acidic oils (Johnson and Hoskins, 1952) have also been considered as possible primary modes of action.

PLANT SUSCEPTIBILITY

Species Differences

There were important differences among plant species in susceptibility to oil injury. Members of the Umbelliferae were notably resistant to acute injury by low-boiling hydrocarbons, so much so that petroleum products have been used as selective herbicides in, for example, carrot crops. Crafts and Reiber (1948) found that sowthistle, wild lettuce, and chickweed were quite resistant; pigweed, goosefoot, lambs' quarter, flax, and onion were intermediate, and grasses, fiddleneck, and mustard were very susceptible to oil injury. Conifers were almost as resistant as carrots, whereas several other species possessing oil ducts were moderately resistant (Havis, 1950). Other species have been compared by Currier (1951), Minshall (1961), and Boyles (1975). Differences in sensitivity may arise because tissues resist penetration of the oils by means of thick cuticles or lack of stomata. In the Umbelliferae, resistance has been shown to be a characteristic of the cells (Currier, 1951; Dallyn, 1953; Minshall, 1961; Boyles, 1976) and may be due to differences in the plasma membrane. Umbelliferae were not selectively resistant to chronic injury by heavy oils (Crafts and Reiber, 1948) and emulsification in water reduced or destroyed selectivity (Dallyn, 1953; Havis, 1950).

Environmental Effects

Environmental conditions may affect the action of petroleum products in various ways. . Injury was reduced when stomata were closed, as in the dark (Van Overbeek and Blondeau, 1954; Dallyn, 1953). In addition to its effect on stomatal opening, light accelerates the bleaching (chlorophyll destruction) of injured tissue, and may also stimulate the oxidation of oils on the plant, increasing their toxicity (Cuille and Blanchet, 1958). Gudin et al. (1976), however, obtained more scorch symptoms on tomato plants under low-light conditions.

High temperatures during oil spraying also led to more injury (Crafts and Reiber, 1948; Cuille and Blanchet, 1958; Calpouzos, 1969), although Dallyn (1953) found little temperature effect. Temperature affects evaporation rate and viscosity of the oil, as well as the physiology of the plant. A few reports suggest that susceptibility can be varied by exposing plants to different growing conditions before the oil treatment (Dallyn, 1953; Minshall, 1961). Currier and Peoples (1954) found that younger carrot plants were slightly more susceptible than older plants.

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